ISOLATION AND CHARACTERIZATION OF ARSENIC-RESISTANT BACTERIAL STRAINS FROM MAMMALIAN GUT

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IN
MICROBIOLOGY

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The entire dissertation work has been done under my supervision and this work has not been submitted elsewhere for a degree.

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I hereby declare that the work which is being presented in this dissertation entitled "**Isolation and Characterization of Arsenic-resistant Bacterial Populations from Mammalian Gut**" in partial fulfillment of the requirements for the award of the degree of **Master of Science in Microbiology** is an authentic record of my own work carried out during the period from **January 2025** to **May 2025** under the supervision of **Dr. Souryadeep Mukherjee, Department of Life Sciences, Presidency University.**

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ABSTRACT

Arsenic is found to be one of the ubiquitous heavy metal contaminant in the environment particularly in groundwater levels and also in food chains. Several studies have been conducted to investigate the toxic effects of arsenic on physiological health of host's body and the emergence of arsenic-resistant bacterial strains, mostly by analyzing fecal matter, but the study on mammalian gut bacteria impacted by arsenic toxicity is less understood. This study focuses on isolating and characterizing bacterial strains from the gut of Wistar rats exhibiting resistance to arenic. The rats were subjected to sub-chronic doses of Sodium-arsenite (NaAsO2) to isolate the bacterial strains resistant to arsenic and further biochemical and molecular characterization were carried out. Atomic Absorption Spectroscopy (AAS) method was applied to confirm arsenic accumulation in the mammalian gut. A total 0f 59 arsenic-resistant bacterial isolates were purified and studied experimentally for further analysis. Along with biochemical and molecular characterization, antibiotic susceptibility test was performed to investigate the potential link between arsenic exposure and antibiotic resistance. The treated group exhibited a marked rise in the proportion of arsenic-resistant bacterial strains compared to the control group. Additionally, bacteria isolated from the arsenic-exposed group demonstrated resistance to all 27 antibiotics tested, while those from the control group showed resistance to only 16. The Multiple Antibiotic Resistance (MAR) index was found to be three times higher for the treated group due to arsenic exposure in comparison to control group of rats. This study underscores the combined rik of arsenic exposure in mammals, demonstrating its role in disrupting gut microbiota balance and promoting the emergence of antibiotic-resistance genes, both of which significantly damages host health. The arsenic-resistant bacterial strains identified in this study could serve as useful indicators of environmental contamination and may hold promise for future applications in bioremediation strategies.

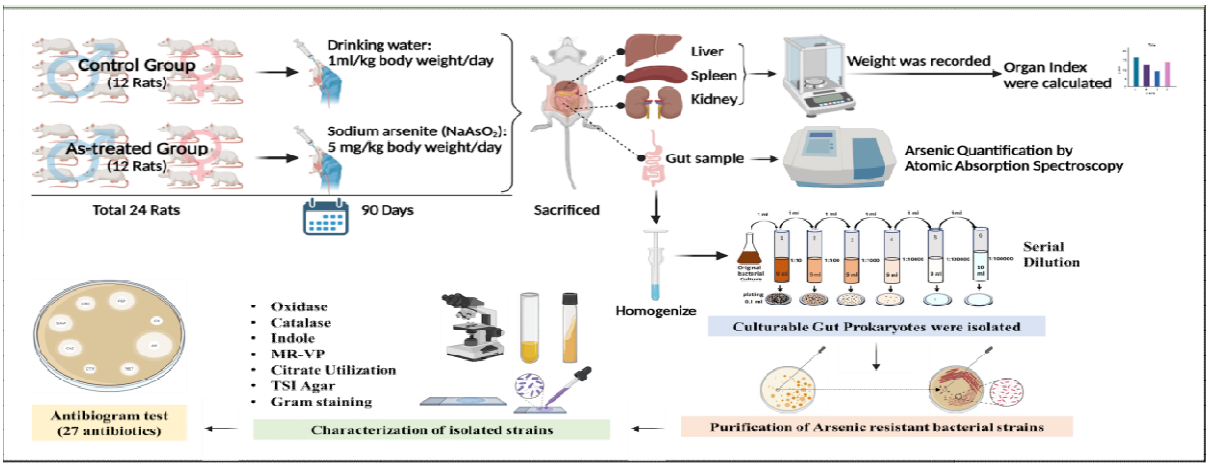


Fig 1: Graphical Representation of the Experimental Workflow

Keywords: Heavy metal, Arsenic, Wistar rats, Sodium arsenite, Dysbiosis, Atomic Absorption Spectroscopy, Antibiotic-resistance, Multiple Antibiotic Resistance index

Abbreviations: **$NaAsO_2$** - Sodium Arsenite ; **AAS** - Atomic Absorption Spectroscopy ; **MAR** - Multiple Antibiotic Resistance

INTRODUCTION

GUT MICROBIOTA AND THEIR ROLE IN HOST HEALTH

Microbiota consists of the wide variety of microorganisms- such as bacteria, fungi. and archae, that inhabit the human body. These microbial populations, particularly present in the lower gut, have evolved along with humans, providing a range of health advantages. [Afzaal, M. et al., (2022) ; AboNahas, H. H. et al., (2022)] When combined with the host's own DNA, the genetic material of these microbes constitutes the microbiome. [Giambò. et al., (2022)] Colonization of the gut microbiota begins in the womb and continues to mature across an individual's lifespan, influenced by factors such as age, health, environment, and antibiotic exposure. The gut microbiota primarily consists of bacterial phyla like *Firmicutes*, *Bacteroides*, and *Proteobacteria*, and also viruses, particularly bacteriophages, also fungi such as *Mycobacteria*. [Huttenhower C. et al., (2012)] Due to its vital role in functions like digestion, metabolism, immune modulation, and mental health, this microbial community is often referred to as the body's "hidden organ" or "super organism". [Xiang-qian Wang. et al., (2018)] Generally, the relationship between gut microbes and their human host is either mutualistic or neutral. [V. Robles Alonso and F. Guarner. et al., (2013)] Although the adult gut microbiota tends to maintain stability, its composition can be significantly influenced by genetics, diet, medication, illness, and environmental toxins such as heavy metals, all of which can disturb microbial balance and negatively impact health.[Gomaa, E. Z. (2020)]

Research using advanced molecular tools aims to uncover how these variables affect microbial structure and contributes to disease. Germ-free animal models—raised in sterile conditions—have provided insight into the microbiota's essential role, revealing compromised immune systems and poor nutrient uptake in the absence of gut microbes. [Bäckhed, F. et al. (2004) ; Round, J. L., & Mazmanian, S. K. (2009)] These microorganisms break down indigestible dietary fibres to generate Short-Chain Fatty Acids (SCFAs) such a butyrate, which play akey role supporting colon health, preserving an acidic gut environment and inhibiting the growth of harmful pathogens. [E. Alvarez-Curto. et al., (2016); A. Woting and M. Blaut. et al., (2016)] Furthermore, gut microbiota plays a crucial role in modulating immune function, especially within the intestinal lining. Around 80% of the body's IgA antibodies originate in this region. They play a key role in immune activation and inflammation, minimizes allergic and hypersensitive responses, and enhancing oral tolerance via action of regulatory T cells. [S. Hapfelmeier. et al., (2010) ; Duboc. H. (2012)]They also support epithelial cell growth and influence neuroendocrine communication, contributing to systemic physiological well-being. [Mestecky, J. et al., (1999)]

FACTORS ALTERING THE STEADY STATE OF GUT MICROBIOTA

An imbalance or disruption in the structured microbial community of the human gut, known as dysbiosis, is associated with various disease conditions. Such imbalances may occur due to loss of beneficial bacteria, overgrowth of pathogenic species or reduction in microbial diversity. [H. Brussow. (2019)] Dysbiosis is not simply a microbial imbalance, it also has profound effects promoting to the pathogenesis of several chronic diseases. It can result in increased gut permeability (leaky gut), trigger chronic inflammation and impair nutrient metabolism. [Microbial Biotechnology (2020); Bischoff, S. C., et al. (2014)]

The leading factors that pave the path towards Dysbiosis are :

- **Dietary Patterns** – Diet is the most influential factor affecting the gut microflora. Diets full of fiber- derived from sources like vegetables, fruits and legumes- are fermented by microbes present in the gut, leading to the production of key Short-Chian Fatty Acids(SCFAs) such as propionate, butyrate, and acetate. [Makki, K., et al., (2018)] This fermentation process supports the growth of beneficial bacterial, including *Lactobacillus* and *Bifidobacteria* species, within the gut. [Reddy BS. et al., (1975)] High fat, sugar and highly processed diets on the other hand can reduce the SCFA production, impairs microbial diversity and also promotes the passage of pathogenic bacteria which leads to inflammation and metabolic disorders. Protein rich diet especially from animals leads to increased fermentation in the colon producing harmful compounds such as ammonia, hydrogen sulphide and phenols. These compounds if present can lead to inflammation and increased cancer risk. [Kinross et al., (2011)]
- **Antibiotic Use** - The commonly known life-saving drugs can be a disadvantage in maintaining the homeostatis of the gut. Broad spectrum antibiotics are known to suppress the growth of both pathogenic as well as beneficial commensal bacteria. The loss of the commensal bacteria from the gut allows the entry of pathogenic species such as *Clostridium difficile* causing infection and coalitis. [Jandhyala. SM. et al., (2015)] Both short-term and arly exposure can negatively impact the normal development of gut microbes, potentially raising risk of obesity, allergies, and autoimmune disorders later in life. [H. Neuman. et al., (2018) ; Francino, M. (2016)]
- **Environmental Exposures** – Excessive cleanliness and reduced exposure to microbial activity during infancy may lead to poor immune tolerance and hence immune-mediated disorders. Continued exposure to environmental pollutants and heavy metals leads to increased gut permeability and trigger systemic inflammation. [Kimberley Parkin. et al., (2019) ; Jin Y. et al. (2016) ; Zhang Q. et al., (2013)]

- **Stress and Mental Health** – Psychological stress significantly impacts gut health because of the bi-directional transmission between the gut and the brain. The gut microbes and the central nervous system influence each other, so any disruption in the microbial balance can directly impact the nervous system. [Borros M Arneth. et al., (2018)] Stress hormones like cortisol and norepinephrine can alter the gut environment and reduces commensal populations. [Crumeyrolle-Arias. M. et al., (2014) ; Wang Y. et al., (2018)] Dysbiosis of gut microbes influences anxiety and mood disorders due to the disruptions of the pathways that produce neurotransmitters like GABA and serotonin. [Strandwitz., P. (2018)]
- **Aging and Immunosenescence** – Aged adults have lower microbial diversity in their gut due to age-related immune decline increasing the risk of infections, chronic inflammations and metabolic disorders. Due to their increased use of medications their gut microbiota often gets disturbed promoting disorders. [Li H. et al., (2022) ; Zhang, Z. J. et al., (2025)]

- **Medical conditions and therapies** – Inflammatory Bowel Disease (IBD), arises from as well as contributes to imbalances in the gut microbiota. Such patients have lower levels of anti-inflammatory bacteria and increased harmful ones such as Escherichia coli.[Frank, D. N. et al. (2007)] Dysbiosis is commonly associated with conditions like insulin resistance, obesity, and Type 2 Diabetes, often resulting from altered energy processing, Short-Chain Fatty Acid (SCFA) synthesis, and systemic inflammation. Additionally, cancer treatments can greatly disturb the gut microbial composition, causing a higher risk of gastrointestinal isues, infections and decreased effectiveness of therapy. [P. D. Cani. et al., (2007)]

IMPACT OF DIFFERENT ENVIRONMENTAL POLLUTANTS AND OTHER FACTORS ON GUT DYSBIOSIS

Human health is sculpted by a dynamic and cumulative range of environmental exposures from conception through the entire lifespan, collectively referred to as the "exposome". These exposures can be air, water, food pollutants or lifestyle-related factors which in turn have a direct impact on the human gut altering the gut microbiota. Over thelast 100 years, the world has been significantly impacted with an increased surge in urbanization, industrialization and agricultural intensification. Coincidentally, the human population has become more prone to chronic inflammatory, metabolic and neurological disorders- all of these are lined to alterations of the gut microbiome.

- **HEAVY METALS (HM)** – These are high atomic weight and high density naturally occurring chemical that are generally shuttled with particulate matter in urban areas and then passes to water and soil environments.[Tchounwou. PB. et al. (2012)] According to recent research, these harmful compounds are continuously building up in our tissues and significantly altering the activity and stable state of the gut microbiota. The increased abundance of heavy metals like lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), and chromium (Cr) is largely attributed to mining, industrial processes, the use of pesticides, and fossil fuel combustion. [Rosenfeld, C. S. (2017)] These toxic elements enter our body using the passage of inhalation, ingestion and absorption and also get accumulated along the food chain. Exposure to environmental toxins can alter microbial diversity, abundance, and metabolite synthesis since the gut microbiota is extremely susceptible to these compounds. [Guan. (2019) ; Balali-Mood. et al., (2021)]

HEAVY METALS	SOURCES	PROPERTIES	MECHANISM OF ACTION	HEALTH IMPLICATIONS
CADMIUM(Cd)	Industrial emissions - Phosphate fertilizers - Cigarette smoke - Contaminated food (grains, leafy vegetables)	- Non-essential, toxic - Long biological half-life (~10–30 years) - Accumulations in liver and kidneys results in oxidative stress of gut microbes	Induces oxidative stress in gut microbes - Disrupts epithelial barrier integrity - Alters SCFA production - Reduces mucosal immunity	- Increases gut permeability (leaky gut) - Linked to IBD and colon cancer - Disrupts nutrient absorption - May affect neurodevelopment via gut-brain axis
LEAD(Pb)	- Lead-based paints - Plumbing systems - Soil contamination - Battery and ammunition industries	- Highly toxic - Bioaccumulates in bones and soft tissue - No safe exposure level	- Inhibits tight junction proteins - Triggers immune activation in mucosa - Disturbs gut-brain communication	- Linked to neurotoxicity and behavioral disorders(e.g.,ADHD) - Triggers systemic inflammation - Increases risk of metabolic syndrome
ARSENIC(As)	- Contaminated	- Exists in	- Microbial	- Promotes liver

	groundwater - Rice, seafood - Industrial waste - Wood preservatives	organic/inorganic forms - Inorganic arsenic is highly toxic - Carcinogenic (Group 1 IARC)	metabolism of Arsenic in the gut is hindered - Disrupts host detoxification processes - Modifies gut immune responses - Encourages dysbiotic configurations	and colon carcinogenesis - Leads to insulin resistance and diabetes - Weakens host immunity - Contributes to chronic gut inflammation
MERCURY(Hg)	- Fish (methylmercury) - Coal combustion - Dental amalgams - Mining operations	- Neurotoxic (especially methylmercury) - Lipophilic and bioaccumulative - Crosses blood-brain and placental barriers	- Disrupts DNA replication in microbes - Alters gut enzymatic activity - Promotes inflammatory cytokine release - Interferes with gut-brain axis	- Linked to autism spectrum disorders - Triggers neuroinflammation - Promotes oxidative damage - Causes gastrointestinal distress
CHROMIUM(Cr)	- Leather tanning - Electroplating - Industrial dyes and pigments - Steel manufacturing	- Cr(III) is essential in small amounts - Cr(VI) is highly toxic and carcinogenic - Soluble and mobile in water	- Induces epithelial cell apoptosis - Increases intestinal permeability - Causes ROS generation in microbiota - Impairs anti-inflammatory signaling	- May cause colorectal carcinogenesis - Leads to mucosal inflammation - Increases susceptibility to infections - Interferes with glucose metabolism

Table 1: Some Common Heavy Metals

- **AGRICULTURAL CHEMICALS AND PESTICIDES** – Recent studies have shed light on the tremendous increase in the use of agricultural chemicals like pesticides, herbicides, insecticides to maximize crop yield and their impact on the bi-directional relationship between gut-microbiota and pesticides. [Giambò et al., (2022) ; Claus SP. et al., (2016)] Although some of the pesticide residues are known to be enzimatically metabolized by the gut-microbiota after their ingestion and absorption partially reducing their toxicity. Still, on the other hand, the compounds produced after their metabolism or the pesticides themselves may have toxic effects on our gut microbial populations. Glyphosate, the ubiquitous pesticide used for controlling weeds in both conventional as well as genetically-modified crops. Its widespread use has led to accumulation in soil, water and even food making human exposure unavoidable. [Syromyatnikov MY. et al., (2020)] Continuous exposure to this pesticide has led to alteration of the gut microbial composition by directly attacking the shikimate pathway which is present in the microbes but absent in humans. [Mesnage, R., & Antoniou, M. N. (2017)] This result in a decline in the beneficial microbial flora such as *Lactobacillus* and *Clostridium* while promoting the proliferation of pathogenic and pro-inflammatory taxa belonging to the *Proteobacteria* phylum. [Claus SP. et al., (2016)] A decrease in beneficial bacterial populations prevent the production of short-

chain fatty acids(SCFAs), impairing vitamin synthesisthus compromising the maintenance of the mucosal barrier. Chlorpyrifos(Cpf) I another widely used pesticide used for fruit and vegetable crops. Chronic exposure to Cpf has led to increase in populations of phylum *Proteobacteria* often associated with inflammation. [Joly Condette. C. (2015)] This is also accompanied with a decline in *Bacteroides*, *Lactobacillus* and *Bifidobacterium* populations, all of which are essential for gut health and also causes disruption of epithelial barrier function. [Claus SP. et al., (2016)]

- **MICROPLASTICS AND NANOPLASTICS** – Microplastics are plastic particles that are microscopic in size, measuring less than five millimeters. They are either intentionally manufactured for use in cleaning agents and cosmetics or they can arise from degradation of larger plastic debris.[Rosenfeld CS. (2017) ; Hadrup. et al., (2012)] Their small size makes their presence ubiquitous as they are more prone to be ingested by various organisms through contaminated food, water and air. Their potential to bind and carry other environmental pollutants, such as phthalates, heavy metals, and microbial pathogens also pose a greater risk. [Rosenfeld CS. (2017)] Microplastics can physically damage the gut-lining, leading to leaky gut promoting systemic inflammation and microbial imbalance. These can also lead to chemical leaching of toxic additives like bisphenol(BPA) or phthalates disrupting host-microbe interactions.[Song. et al., (2015)] Ingested microplastics can also stimulate immune cells to release pro-inflammatory cytokines creating an environment hostile to beneficial microbes. They have also been linked to elevated production of reactive oxygen species (ROS), which inhibits microbial growth by damaging their DNA.[Zhu D. et al., (2018)] Alongside, nanoparticles such as titanium dioxide, silicon dioxide used in food additives are also consumed daily. Nanoparticles trigger gut inflammation and increase cytokine production, fostering an environment favorable to pro-inflammatory microbes. [National Institute of Environmental Health Sciences. Bisphenol A (BPA). (2021) ; Cheng Y. et al., (2021)]

- **ENDOCRINE DISRUPTORS** - These are environmental chemicals known to interfere with hormonal regulation and signaling. The gut microbiota serves both as a target and a mediator of Endocrine Disruptor's effects. [Liu. et al., (2016)] Firstly, EDs can impair microbial metabolism by altering gene expression and enzyme production. This imbalance impairs the bacteria's ability to break down dietary fibres and other non-digestible compounds into short-chain fatty acids(SCFAs). By increasing insulin sensitivity and influencing liver glucose synthesis through specific G-protein-coupled receptors, these SCFAs are crucial for controlling glucose balance. [Lai. et al., (2016)] Moreover, gut microbes can metabolize EDs into derivative compounds with different biological activities. For instance, dichlorodiphenyltrichloroethane (DDT), a well-known pesticide and ED,can be biotransformed into dichlorodiphenyldichloroethane (DDD) by intestinal bacteria. These changes may lessen or increase the toxicity of the original chemical, depending on the metabolic pathway involved, and the microbial composition. [Rosenfeld CS. (2017) ; Liu. et al., (2016); Lai. et al., (2016)]

- **ANTIBIOTICS** - Since their discovery, antibiotics have been treated as one of the most significant medical advancements that saved millions of lives by effectively treating bacterial infections. [Modi, S. R. et al., (2014)] Although, their benefits in combating pathogenic bacteria are undeniable, their widespread and injudicious use has raised serious concerns about the beneficial commensal bacterial populations of the gut. Their therapeutic effects are exerted by inhibiting bacterial cell wall synthesis, protein synthesis, DNA replication and other metabolic pathways. [Francino. MP. (2016)] During the exposure of antibiotics both the pathogenic and non-pathogenic bacterial populations become susceptible to their

doses. Beneficial bacterial species such as *Bifidobacterium*, *Lactobacillus* colonies are disrupted by the effects of broad-spectrum antibiotics. Disruptions of these species prevent the processes such as fermentation of indigestible dietary fibres into SCFAs, vitamin synthesis, pathogen resistance, and immune system modulation. [Mai. et al., (2011, 2013)] When these beneficial bacteria are diminished, opportunistic pathogens such as *Clostridioides difficile*, *Escherichia coli*, and *Klebsiella* species may overgrow, leading to infection or inflammation. A notable clinical manifestation is *Clostridioides difficile* infection (CDI), a fatal condition marked by severe diarrhea and colitis, commonly triggered after antibiotic exposure. Extended research suggests that individuals who are regularly exposed to antibiotics, particularly from childhood days, face an increased risk of developing IBD. [Rogers and Aronof. (2016)] The mechanism is thought to involve reduced microbial diversity, impaired mucosal immunity, and increased gut permeability—factors that collectively facilitate intestinal inflammation. Such prolonged subjection to antibiotics have posed a greater risk of increased Antibiotic Resistance genes (ARGs). The human gut which often serves as a pool of ARGs, can eventually contribute to the dissemination of pathogenic species making further treatment processes difficult to commence. [Vanner, (2008) ; Bajinka. et al., (2020)]

ROOT OF ANTIBIOTIC RESISTANCE AND ITS IMPACT ON GUT DYSBIOSIS

Beyond posing a threat to public health, the injudicious use of antibiotics have caused a significant increase in antibiotic resistance, disrupting the stability of the gut microbiota. This resistance develops when bacteria evolve mechanisms to survive antibiotics that once effectively eliminated them. [Cusumano, G. et al., (2025)] Bacteria acquire resistance either inherently or through horizontal gene transfer (HGT), allowing them to rapidly spread resistance traits within microbial communities. [Frost LS. et al., (2005)] Once resistant, these bacteria can proliferate unchecked, making infections increasingly difficult to treat. To withstand antibiotic pressure, bacteria deploy various strategies. These include producing enzymes like β-lactamases that inactivate antibiotics, altering target sites to prevent effective drug binding, reducing drug uptake through changes in membrane permeability, and using efflux pumps to expel antibiotics. [Blair, J. M. A. et al., (2015) ; Nikaido, H. (2009)] Bacteria also form biofilms—protective layers that provide defence to colonies against attack from antibiotics. [Hall, C. W. & Mah, T.-F. (2017)] Genes for such resistance pathways are often carried on plasmids and transposons, which allow them to be shared across various bacterial species. [Francino MP. (2016) ; De La Cochetiere. et al., (2005)]

Several human practices play a pivotal role in proliferation of antibiotic resistance. In healthcare, inappropriate prescriptions—such as using antibiotics for viral infections—are common. In agriculture, antibiotics are used to promote animal growth and prevent disease, facilitating resistance gene transfer to humans through food and the environment. [Pallecchi. et al., (2007)] Pharmaceutical waste also contaminates natural ecosystems, creating hotspots for resistant bacteria. In the gut, antibiotics not only target pathogens but also disrupt beneficial microbes, leading to dysbiosis. [Ghosh. TS. et al., (2013)] Such microbial imbalance can compromise the integrity of the gut barrier, interfere with metabolic processes, and reduce immunological responses. Numerous health problems, including as diabetes, heart disease, obesity, and neurological abnormalities, have been connected to it. The gut serves as a significant reservoir for ARGs, where extended antibiotic use encourages the promotion of resistant bacteria. [Doucet-Populaire. et al., (1991) ; Sommer. et al., (2009)]

ROLE OF DIFFERENT ENVIRONMENTAL POLLUTANTS IN THE EMERGENCE AND SPREAD OF ANTIBIOTIC RESISTANCE

Environmental pollutants are emerging as significant contributors to antibiotic resistance, alongside clinical misuse of antibiotics. Contaminants introduced into soil, water, and air through human activities create selective environments promoting antibiotic resistance, even when antibiotic are not immediately present.[Baker-Austin et al., (2006) ; Kurenbach, B. et al. (2015)]

Pesticides and herbicides, including glyphosate and chlorpyrifos, disrupt bacterial structures and stress responses, promoting mutations and horizontal gene transfer that spread resistance. [Skandalis, N. et al., (2021)] Pharmaceuticals and personal care products (PPCPs), often present in water bodies due to improper disposal and incomplete metabolism, apply selective pressure even at low concentrations, enriching ARGs in microbial populations. [Michael, I. et al. (2013) ; Kolpin, D.W. et al., (2002)] Non-antibiotic drugs such as anti-inflammatory agent and disinfectants can influence bacterial resistance by mechanisms like cross-resitance and co-regulation.[Maurice, C. F., Haiser, H. J., & Turnbaugh, P. J. (2013)] Wastewater treatment plants (WWTPs) are hotspots for resistance development due to high microbial loads and the mixing of pollutants. They often release treated water that still carries resistant bacteria and genes into the environment. [Skandalis, N. et al., (2021) ; Pärnänen. et al., (2019)] Microplastics provide environments for bacterial colonies to attach and biofilms formation, creating habitats known as the "platisphere" that promotes gene exchange and ARG proliferation.[Wang, J. et al. (2021)] Heavy metals like mercury, arsenic, lead, and copper—commonly present in industrial and agricultural waste—can promote the selection of antibiotic resistance genes (ARGs), as these genes are often genetically linked to metal resistance genes on mobile genetic elements.This genetic linkage promotes co-selection, wherein exposure to heavy metals indirectly enriches resistant bacteria populations even in antibiotic-free environments. [Wright, G.D. et al., (2010)]

Environmental stress factors exert selective pressure on bacterial populations, encouraging the survival of resistant strains. Moreover, pollutants frequently cause oxidative stress, increasing bacterial mutation rates and genetic recombination. Many resistance genes are housed on plasmids, integrons, and transposons, facilitating their horizontal transmission. Environmental reservoirs thus play a critical role in the evolution, development, and spread of ARGs. [Faleye, A.C. et al., (2019) ; Chowdhury, S. et al., (2016) ; Wales, A.D. et al., (2015) ; Martinez, (2009) ; Bengtsson-Palme. et al., (2018)]

EFFECTS OF GUT DYSBIOSIS ON HUMAN HEALTH

1. **GASTROINTESTINAL DISORDERS**: Disruption in gut microbiota composition is majorly linked to GIT disorders, including, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD) and colorectal cancer [Parkin, K. et al., (2021)] In IBD,through forms such as ulcerative colitis (UC) or Crohn's disease (CD) – this microbial imbalance activates immune responses and reduces the integrity of the intestinal barrier. [Carding, S. et al., (2015) ; Baumgart DC. et al., (2007)] IBS is linked to reduced microbial diversity and increased gas-producing bacteria. Dysbiosis can promote colorectal cancer through immune suppression and also raises the risk of *Clostridioides difficile* infection post-antibiotic use. Additionally, it disrupts estrogen metabolism, potentially contributing to hormone-related cancers. [Carroll IM. et al., (2010)]

2. **METABOLIC DISORDERS**: Gut microbiota significantly influences energy regulation and metabolism. A lower Bacteroides-to-Firmicutes ratio enhances calorie absorption,

promoting fat storage. [Turnbaugh, P. J. et al., (2006)] Dysbiosis allows lipopolysaccharides to enter the bloodstream, triggering chronic inflammation and insulin resistance, contributing to type 2 diabetes. [Cani, P. D. et al., (2007)] SCFAs regulate appetite, glucose, and fat metabolism, playing a vital role in obesity. [Canfora, E. E. et al., (2015)] Additionally, altered gene expression and increased FIAF activity affect lipid metabolism, as shown in fecal microbiota transplantation and dietary studies. [Bäckhed, F. et al. (2004)]

3. **CENTRAL NERVOUS SYSTEM – RELATED DISORDERS**: The gut-brain axis, also referred to as Hypothalamic-Pituitary-Adrenal (HPA) axis, through a complex network of neurological, hormonal, immune, and metabolic signaling pathways, links the gut microbiota to the central nervous system via the vagus nerve.[Parkin, K. et al., (2021)] Dysbiosis is linked to disorders like anxiety, depression, autism, and neurodegeneration. Altered microbial profiles in Autism Spectrum Disorder (ASD) and reduced SCFA levels can impact neurotransmitter production. [Arneth BM. et al., (2017)] Microbial metabolites like tryptophan and SCFAs affect brain chemistry, while certain bacteria influence mood by modulating stress pathways and GABA receptor expression in the brain. [Rogers GB. et al., (2016) ; Sampson TR. et al., (2015)]

4. **CARDIOVASCULAR DISORDERS AND HYPERTENSION**: Recent studies reveal a strong link between gut dysbiosis and cardiovascular conditions like atherosclerosis and coronary artery disease. Microbial imbalance weakens gut barrier function, allowing LPS to enter circulation, triggering inflammation via TLRs and promoting plaque buildup. [Castellani, V. (2025)] Elevated Trimethylamine-N-Oxide (TMAO) levels, reduced Bile Salt Hydrolaes (BSH) -producing bacteria, and microbial DNA in plaques suggest bacterial involvement. [Tang, W. H. W. et al., (2013) ; Jones, BV. et al. (2008) ; Koren, O. et al. (2011)] Gut microbes also influence blood pressure, with hypertensive individuals showing distinct microbial profiles, especially under high-salt dietary conditions. [Yang, T. et al. (2015)]

5. **IMMUNE SYSTEM ALTERATION AND AUTOIMMUNE DISORDERS**: The healthy growth and control of the immune system depend on the gut bacteria. This equilibrium is upset by dysbiosis, which leads to a rise in pro-inflammatory Th17 cells and a fall in regulatory T cells, ultimately causing chronic inflammation.[Belkaid, Y., & Hand, T. W. (2014)] Increased intestinal permeability allows microbial products like LPS and flagellin to enter the bloodstream, triggering immune activation. This contributes to autoimmune diseases such as T1D, MS, and RA, where altered microbial profiles and immune signaling promote tissue damage through cytokines like TNF-α and IL-6**.** [Winiarska-Mieczan, A. et al., (2022) ; Di Vincenzo, F. et al., (2024)]

INFLUENCE OF HEAVY METALS ON GUT MICROBIOTA

Heavy metals like arsenic (As), cadmium (Cd), lead (Pb), chromium (Cr), and mercury (Hg) are notable for their damaging effects on human health, causing issues like neurotoxicity, compromised immune responses, kidney damage, reproductive problems, skin conditions, vascular damage, and a heightened risk of cancer. [P. Bist, S. Choudhary. (2022)] In recent years, research has increasingly focused on how these metals affect the gut microbiota—a diverse microbial community crucial to human health. Disruption in this microbial ecosystem, termed gut dysbiosis, is linked to a range of chronic illnesses. This chapter explores how heavy metal exposure contributes to gut dysbiosis, focusing on toxicity mechanisms, effects on gut barrier integrity, and subsequent implications for gastrointestinal and systemic health. Additionally, heavy metals are now recognized for their role in promoting antibiotic resistance. They share

resistance pathways with antibiotics—such as efflux pumps, genetic linkage of resistance genes, and similar selective pressures—which can lead to the co-selection and proliferation of multidrug-resistant bacteria. [Berg et al., (2005); Baker-Austin, C. et al. (2006)] Such co-regulatory relationship worsens the worldwide problem of antimicrobial resistance (AMR), making it difficult to treat infections and promoting increased rates of disease and mortality. In addition, heavy metals generate reactive oxygen species (ROS) that harm proteins and DNA, negatively affecting the survival of microbes. [Jomova, K., & Valko, M. (2011)] Studies on animal models have shown that cadmium exposure decreases beneficial bacterial populations like *Lactobacillus* and *Bifidobacterium*, while favoring opportunistic pathogens such as *Escherichia* and *Clostridium*. Arsenic has similarly been shown to reduce microbial diversity and alter the *Firmicutes*-to-*Bacteroidetes* ratio. [Breton, J. et al. (2013)] Overall, heavy metals disrupt microbial balance by eliminating sensitive strains and favoring resistant ones, impairing gut function and health.

Heavy metals exert their effects on dysbiosis through the following pathways :

1. **Oxidative Stress** – Microbial death and altered community structure in the gut often caused by microbial DNA, lipids and protein damage implicated by heavy metals such as Hg and Cd by generating ROS. [Jomova, K., & Valko, M. (2011)]
2. **Membrane Disruption** – Certain metals can disrupt bacterial membrane, hindering ion gradients and nutrient transport which can selectively eliminate sensitive strains. [Nies, D. H. (2003)]
3. **Metal Binding and Chelation** - Metals may sequester essential nutrients like iron or manganese, limiting microbial access and favoring species with high-affinity transporters. [Adhikary, S. et al., (2024)]
4. **Interference with Metabolic Pathways** - Metals can inhibit key enzymes involved in fermentation and respiration, altering the metabolic output of the microbiota, such as SCFA production. [Claus, S. P. et al., (2016)]

A frequently underestimated yet critical consequence of heavy metal exposure is the rise and dissemination of antibiotic-resistant bacteria. This occurrence is primarily due to co-seletion, in which resistance genes for both heavy metals and antibiotics are found together on plasmids, transposons or integrons.[Seiler, C., & Berendonk, T. U. (2012)] Consequently, when bacteria encounter heavy metals, selective pressures promote the survival of strains resistant to both metals and antibiotics, even in the absence of antibiotics. Cross-resistance compounds the problem, as a single mechanism—such as multidrug efflux systems—can shield microbes from both antimicrobial agents and toxic metals. For example, the czc operon, which enables resistance to zinc, cobalt, and cadmium, is also implicated in resistance to β-lactam antibiotics. [Nies, D. H. (2003)] Numerous studies demonstrate a strong correlation between environmental heavy metal contamination and elevated antibiotic resistance gene (ARG) prevalence. Polluted soils and aquatic systems have been shown to harbor higher levels of ARGs like bla, tet, and sul. [Zhu, Y. G. et al. (2013)] This dual threat is amplified by gut dysbiosis, which disrupts vital functions such as immunity, digestion, and metabolism. Chronic, low-level heavy metal exposure may quietly foster microbial imbalance and promote ARG dissemination, highlighting the urgent need for environmental and public health intervention. [Berendonk, T. U. et al. (2015)]

ARSENIC AS A HEAVY METAL

Arsenic is a naturally occurring metalloid, grouped with heavy metals often due to its toxic effects on biological systems. It is prevalent both in organic and inorganic form, the inorganic variants- particularly arsenite (As^{3+}) and arsenate (As^{5+}) – are highly toxic and present serious health risks to humans and animals. [Hughes, M. F. (2002)] Arsenic is omnipresent in the environment and occurs throughout soil, water, air, and different living organisms due to both natural phenomenon and anthropogenic activities. It is the 20th most abundant element in the Earth's crust and can also be found in groundwater sources, and industrialregions. [Naujokas, M. F. et al. (2013) ; Mazumder, (2008)]. Contamination in drinking water due to presence of arsenic has become a major global public health concern, affecting millions worldwide. Arsenic remains stable in anoxic environments but undergoes oxidation when exposed to aerobic conditions. [Nickson, R. T. et al. (2000)]

SOURCES OF ARSENIC

a) Natural Sources [Smedley, P. L., & Kinniburgh, D. G. (2002)] **:**

1. Geological Deposits – Arsenic released from weathered metal ores.
2. Volcanic Activity – Emissions deposit arsenic in soil and water.
3. Hydrothermal Processes – Hot springs leach arsenic into water systems.
4. Erosion and Sedimentation - Eroded rocks spread arsenic in the environment.

b) Anthropogenic Sources [Bhattacharya, P. et al., (2007)] **:**

1. Mining and Smelting – Waste from metal extraction leaches arsenic.
2. Agricultural Use – Pesticides and feed additives contaminate soil.
3. Industrial Effluents – Factories release arsenic into surroundings.
4. Coal Combustion – Burning coal emits arsenic into air.

ARSENIC CONTAMINATION IN GROUNDWATER

Groundwater arsenic pollution poses a significant environmental and public health threat, particularly in regions of South and Southeast Asia. Naturally occurring arsenic, primarily from geological formations, dissolves into aquifers under specific geochemical conditions, such as reducing environments with low oxygen levels. [Shankar, S. et al., (2014)] Research conducted in the Ganges-Brahmaputra Delta of Bangladesh and India revealed that arsenic concentrations in some regions exceed the WHO guideline of 10 µg/L by more than 100 times. [Smedley, P. L. et al., (2002)] Research has indicated that more than 35 million individuals in Bangladesh are exposed to drinking water contaminated with arsenic. Similarly, investigations in West Bengal found high arsenic levels in tubewells, linking prolonged exposure to health problems like skin lesions, cancer, and cardiovascular diseases. [Smith, A. H. et al., (2000) ; Chakraborti, D. et al. (2004)] Contamination is exacerbated by excessive groundwater withdrawal, which alters the redox conditions and mobilizes arsenic. These results highlight the necessity of ongoing surveillance, public education, and affordable arsenic removal methods to protect human health and groundwater quality.

IMPACT OF ARSENIC ON GUT MICROBES

Arsenic is a widespread environmental pollutant found extensively in soil and water, affecting millions globally. Its primary routes of human and animal exposure are through contaminated food and drinking water, with the gastrointestinal tract serving as the first site of contact. Exposure to arsenic has been associated with numerous health issues, such as

cancer, cardiovascular diseases and diabetes. Studies have shown that even low levels of arsenic, such as 10 ppb, can destroy the composition of the gut microbiota and metabolic activity in animals. These disruptions include altered microbial diversity and metabolic functions, impacting energy metabolism, immune signaling, and gut organisation. However, the unpretentious link between microbial alterations and hot health remains unclear. [Lu, K., Abo, R. P. et al., (2014)]

Arsenic exposure has been observed to be co-regulatory in the emergence of antibiotic resistance, according to recent studies. This occurs through co-selection, where mobile genetic elements such as plasmids carry genees that provide resistance to both antibiotics and heavy metals. These findings align with animal studies demonstrating similar gene enrichments in arsenic-exposed microbiomes. [Seiler, C., & Berendonk, T. U. (2012)] Nutritional factors, such as zinc and iron levels, also influence arsenic's impact on gut microbes, highlighting the complexity of these interactions. [Chi, L. et al., (2018)] The effects of arsenic vary across studies, influenced by exposure dose, host species, sex, and dietary status. Despite inconsistencies in microbial composition changes, the role of arsenic in promoting resistance genes is increasingly evident.

INFLUENCE OF HEAVY METALS PROMOTING ANTIBIOTIC RESISTANCE

The simultaneous occurrence of antibiotics and heavy metals in settings like animal guts, poultry operations, and agricultural lands is becoming a major issue for both environmental and public health. In India, livestock feed commonly includes both arsenic and antibiotics to promote growth and disease prevention, leading to dual exposure of gut microbes. Additionally, poultry manure used as fertilizer further contaminates the soil with these toxicants. [Zhu, Y.-G., Johnson. et al., (2013)] While antibiotics degrade faster, their constant introduction into ecosystems renders them “pseudopersistent.” Unlike other pollutants, heavy metals do not break down and can persist in the environment for long durations, raising serious concerns about their cumulative effect on the spread of antibiotic resistance. [Kümmerer, K. (2009)]

In developing nations, the groundwater contamination with heavy metals—often a primary source of drinking water—poses an added risk, as prolonged exposure through water and food may lead to toxic effects and leads to the occurrence of antibiotic-resistant bacterial strains. [Kumar, M. et al., (2009)] Research indicates that exposure to both antibiotics and heavy metals can accelerate the emergence of multidrug-resistant strains. Metals such as zinc, copper, cadmium, and nickel have been strongly associated with increased antibiotic resistance. For instance, studies in India reveal elevated resistance gene levels in river systems contaminated with heavy metals, with poultry farms being significant contributors. [Vats, P. et al., (2022)] Heavy metals promote resistance through processes such as co-regulation, co-selection, cross-resistance. In co-seelction, genes that provide resistance to both heavy metals and antibiotics are carried on the ame mobile genetic elements, allowing bacteria to survive even when exposed only to metals. Cross-resistance arises when shared mechanisms, like efflux pumps, defend against both threats—e.g., the CzcCBA pump in *Pseudomonas aeruginosa*. Co-regulation involves common regulatory proteins that activate multiple resistance genes under stress. [Baker-Austin, C. et al., (2006)] Collectively, these mechanisms allow bacteria to endure in hostile environments, facilitating the spread of resistance and amplifying the risks posed by environmental contamination with heavy metals.

REVIEW OF LITERATURE

The increasing abundance of heavy metals in the urban areas reaches the water and soil. Numerous in vitro and human investigations have shown that heavy metal exposure can alter the gut microbiota's diversity and makeup. Furthermore, by supporting metabolic oxidation or reduction activities in the gastrointestinal tract, the gut microbiota can regulate metal toxicity. However, heavy metals induce oxidative stress, disrupting the gut barrier and hampering the healthy microbiome, leading to dysbiosis. This alteration can increase metal toxicity and oxidative stress, which often play a key role in the development of psychiatric disorders. [(Kholodov AS et al. 2021; Wu N et al. 2020; Giambò, F et al. 2021; Rosenfeld, C.S 2017)].

Heavy metals, particularly in their bioavailable forms, exert stress on bacterial populations, and metal contamination can intensify resistance across various environments. Bacteria develop resistance to metals through multiple mechanisms, including mutations in chromosomal or plasmid DNA, alterations in metal uptake pathways, or the acquisition of resistance genes carried on mobile genetic elements (MGEs). These diverse strategies enable bacteria to adapt and survive in metal-polluted conditions, often contributing to the broader issue of antimicrobial resistance in both natural and human-influenced settings [(Giller et al., 1998; Bruins et al., 2000)]

In certain natural environments rich in microbial diversity, the simultaneous presence of heavy metals and antibiotics encourages the appearance of antibiotic resistance, sometimes leading to multidrug resistance. To show an example, microbial resistance is often shown to be enhanced by combined exposure of zinc and oxytetracycline in sludge bioreactors. Similarly, copper in agricultural soil can select for copper resistance while also co-selecting for resistance to antibiotics like ampicillin, chloramphenicol, and tetracycline. Nickel and cadmium have also been found to increase resistance to unrelated antibiotics. This may result from the selective enrichment of native bacteria already carrying resistance genes or the induction of resistance in previously sensitive strains. Although a strong correlation exists between ARG presence and metal/antibiotic concentrations, limited studies have confirmed whether metals directly induce resistance in sensitive bacteria. [(Baker-Austin, C. et al. 2006; Peltier, E. et al. 2010; Berg, J.et al. 2005; Stepanauskas, R. et al. 2006; Knapp, C.W. et al. 2011)]

Overuse of antibiotics can caue dysbiosis, a disorder which disorients the stable condition of the gut microbiota, and microbial resistance. Numerous illnesses, including autoimmune diseases, gastrointestinal disorders, infections, allergies, asthma, arthritis, obesity, and cancer, have been linked to this disruption. Furthermore, psychological and mental health issues have also been connected to alterations in the gut microbiota brought on by overuse of antibiotics. [(Cardetti et al. 2020; Helaly et al. 2019)]

Antibiotic resistance emerges as worldwide health concern, partly fueled by a 65% rise in antibiotic use between 2000 and 2015, especially in low- and middle-income countries. Each year, antibiotic resistant infections result in tens of thousands of death in the US and Europe, with estimates suggesting that by 2050, they could cause up to 10 million deaths globally. In China, resistance against antibiotics has surged due to widespread antibiotic use in livestock. Easy access and misuse of antibiotics fuel resistance, making infections harder and costlier to treat. [(Klein et al., 2018; WHO, 2018; WHO, 2019; Sugden et al., 2016; Van Boeckel et al., 2015)]

As early as the 1960s, researchers began reporting links between heavy metal exposure and resistance to multiple antibiotics. Subsequent studies have confirmed that HMs also act as a selection pressure for ARGs. Laboratory and field studies show that exposure to metals like zinc and copper can induce antibiotic resistance, even at subtoxic or subinhibitory concentrations, through mechanisms like cross-resistance, mutagenesis, and horizontal gene transfer. This poses a serious health risk due to environmental contamination. [(Moore, 1960; Richmond & John, 1964; Bhattacharya et al., 2000; Peltier et al., 2010; Pathak & Gopal, 2005)]

Excessive and inappropriate use of antibiotic has been identified as a key factor in the rise of antibiotic-resistant bacteria (ARB), mainly through the co-selection of ARGs within microbial communities. Although antibiotics are the primary drivers of ARG emergence and dissemination, heavy metals also significantly contribute as co-selective agents, promoting resistance even in settings where antibiotics are not preent. Arsenic (As), although the third most common heavy metal pollutant, can exert a stronger selection pressure on ARGs than cadmium (Cd). Inorganic arsenic (iAs) contamination in drinking water has been widely reported and is a significant threat to public health. iAs exposure disrupts microbiome composition, contributing to gut and oral dysbiosis. Despite growing evidence, the toxic effects of iAs on gut prokaryotes and their antibiotic resistance mechanisms remain inadequately understood. This study aims to address the lack of in-vivo research on HM-induced changes in gut bacterial resistance. Using arsenic shock loading as a model, the study investigates the resulting shifts in bacterial diversity, abundance, and ARG distribution in mammalian guts. It also builds on prior environmental studies in India, aiming to fill critical knowledge gaps. [(Morita et al., 2019; Yang et al., 2019; Parrone et al., 2020; Chouhan & Flora, 2010; Zhang et al., 2020; Lu et al., 2014; Tang et al., 2020; Singh et al., 2023; Alam et al., 2022)]

SCIENTIFIC RATIONALE OF THE STUDY

Arsenic is a widespread environmental pollutant that poses serious health risks, particularly in regions like India where groundwater contamination is common. While extensive research has examined the impact of heavy metals on environmental microbes, limited studies have explored arsenic's effects on gut microbiota. [(Klein et al., 2018; WHO, 2018] Ongoing researches suggest that arsenic not only hampers the composition of gut microbiota but also plays a role in the evolution of ARB by promoting the co-selection of resistance geens.[(Moore, 1960; Richmond & John, 1964; Bhattacharya et al., 2000] Most existing studies rely on fecal samples, which may not truly represent the gut's microbial diversity. There is a lack of in-depth in-vivo studies focused on isolating and identifying arsenic-resistant gut bacteria.

This research aims to extract and characterize arsenic-resistant bacterial species from mammalian gut microbiota to better understand their diversity, resistance mechanisms, and potential health implications. The findings could offer crucial insights into the ecological and clinical risks of arsenic exposure in humans and aid in addressing related public health challenges.

OBJECTIVES OF THE PRESENT STUDY

i. Preparation of animal models keeping vehicular control

ii. Extraction and analysis of bacterial strains resistant to arsenic

iii. Biochemical and molecular characterization of arsenic-resistant bacterial strains

iv. Determination of MAR index using antibiotic-susceptibility test

MATERIALS AND METHODOLOGY

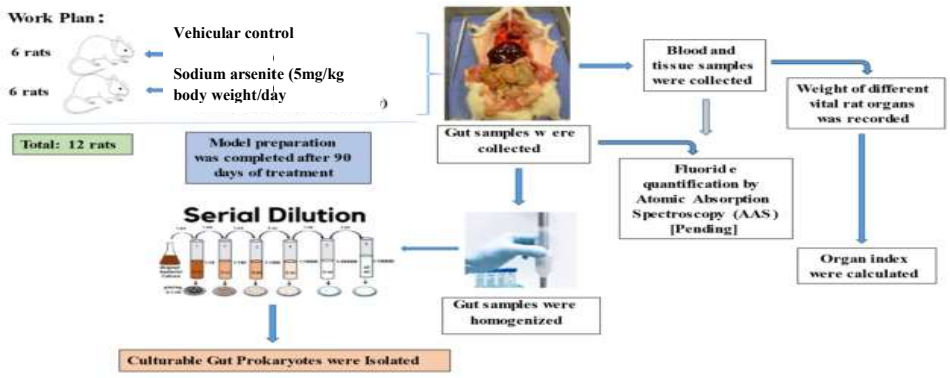

Work Plan :
6 rats
6 rats
Vehicular control
Sodium arsenite (5mg/kg body weight/day
Total: 12 rats
Model preparation was completed after 90 days of treatment
Gut samples w ere collected
Blood and tissue samples were collected
Weight of different vital rat organs was recorded
Fluorid e quantification by Atomic Absorption Spectroscopy (AAS) [Pending]
Organ index were calculated
Serial Dilution
Gut samples were homogenized
Culturable Gut Prokaryotes were Isolated

Fig 2: Graphical Representation of the Processes and Techniques Applied for this Study

1) Preparing animal models keeping vehicular control

- **Selection and Design of the Animal Model:** Toxicological studies that aim at understanding the effects of harmful agents like arsenic on the gut microbiota generally utilizes rodents, particularly rats as the animal model. They are observed to be excellent subjects while examining the toxic effects on human health, due to their shared physiological and genetic similarities to humans. Studies confirm that both rodents and humans possess comparable gastrointestinal microbiota, validating the use of rodent models for drawing meaningful conclusions relevant to humans. These animal models allow for tightly controlled laboratory settings, enabling researchers to systematically manage factors such as diet, environment, and contaminant exposure to assess microbial changes.

  In particular, standardized rodent strains like adult Wistar rats are frequently employed to ensure experimental uniformity, reducing inter-individual variability and thus improving the reliability of results regarding arsenic's impact on gut flora Wistar rats are favored due to their moderate size, ease of handling, and relatively low care requirements, which make them highly practical for extended experimental studies.

These qualities render Wistar rats especially suitable for long-term investigations into how chronic arsenic exposure influences gut health and microbial diversity.

- **Experimental Design:** Adult Wistar rats (*Rattus norvegicus*) were procured from a certified breeder and their use was authorized by the Animal Ethical Committee of Presidency University, Kolkata (Approval No: PU/IAEC/SM/52). A total of 12 rats, including both males and females with an average body weight of approximately 120 grams, were initially housed in polycarbonate cages located in the Quarantine Room of the Animal House Facility, Department of Life Sciences, Presidency University.

  The rats were maintained under standard laboratory conditions, with temperature controlled at $22 \pm 2°C$ and relative humidity stabilized at $45 \pm 5\%$. A consistent 12-hour light/dark cycle was upheld. They were provided unrestricted access to fresh drinking water and a well-balanced pellet-based diet.(see Table 2 for composition).

  Following a two-week quarantine period to ensure the animals were in good health and free from any pre-existing conditions, only those that met specific health criteria were selected for the study. These selected rats were then relocated to the designated Rat Room within the university's animal facility for the main phase of the experiment. This step-by-step protocol ensured the welfare of the animals while also maintaining the scientific rigor and reliability of the experimental outcomes.

SL NO.	INGREDIENTS	TOTAL AMOUNT (in gm)	CARBOHYDRATE	PROTEIN	LIPID	MINERALS AND VITAMINS
1	**Wheat flour**	1500	1155	165	30	150
2	**Roasted Bengal gram flour**	1000	650	260	60	30
3	**Sucrose**	750	748.5	0.75	0.75	0
4	**Casein**	200	4	190	0	6
5	**Refined groundnut oil**	150	0	0	150	0
6	**Salt mixture**	50	0	0	0	50
	TOTAL(in gm)	3650	2557.5	615.75	240.75	236
	PRSENT%		70.068	16.87	6.596	6.466
	EXPECTED%		71	18	7	4

Table 2: Composition of Rat Diet

- **Treatment Procedure:** For this experiment, 12 Wistar rats were acquired and randomly divided into two experimental groups. Group I, designated as the control group, included 6 male and 6 female rats. Group II consisted of an equal number of male and female rats and was subjected to treatment with Sodium Arsenite ($NaAsO_2$). Both groups were closely monitored throughout the experimental period to assess any changes in health and behavior resulting from the treatment. To simulate sub-chronic exposure, Group II rats were exposed to oral administration of $NaAsO_2$ at a dose of 5 mg/kg body weight daily for 90 days. The dosage was applied based on previous research data and represents

approximately 12% of the compound's LD_{50} (oral administration). The chosen concentration also reflects the highest levels of arsenic contamination detected in certain areas of West Bengal. [(Kumar Sharma et al., 2021; 2006; AAT Bioquest, 2023; Mehrzadi et al., 2021; Bashir et al.)]

To ensure relevance and scientific accuracy, the human equivalent dose was carefully converted to a rat-specific sub-chronic dosage. This conversion was guided by Central Ground Water Board according to published toxicological standards and contamination data provided by India's Ministry of Water Resources (2009), along with findings from Datta et al. (2014). This methodical dosing approach helped ensure experimental safety while maintaining relevance to real-world environmental exposure scenarios.

- **Tissue Collection:** The animals were humanely sacrificed, following the 90-day treatment period, using Ketamine administered at a dose of 80 mg/kg body weight, in accordance with ethical guidelines, as recommended by Bhatia et al. (2022). After euthanasia, biological samples were collected for further examination. Approximately 2 ml of whole blood was collected from each rat, and segments of the gastrointestinal tract were excised for detailed analysis.

  Subsequent microbiological experiments involving mammalian gut bacterial isolates were conducted under biosafety cabinet conditions, adhering strictly to all relevant safety and procedural protocols. To assess arsenic accumulation, the levels of arsenic (As) in the gut, blood, and various tissues from both control and treated groups were quantified using atomic absorption spectroscopy (AAS), following standardized analytical methodologies.

➢ Arsenic quantification from gut samples by Atomic Absorption Spectroscopy (AAS)

Experiments were done to prepare gut samples for AAS:

- 0.2 g gut sample from different model animals were taken and 5 ml 1X PBS buffer was added to it.
- Then it was washed using a magnetic stirrer for 2 minutes.
- Next PBS was decanted and Milli Pore water was added.
- It was again stirred with the magnetic stirrer for 1 minute to wash out the remaining PBS.
- Tissue was extracted and blotted on blotting paper.
- Then the tissue was placed on a glass petri plate and kept in a Hot air oven for drying.
- 1:1 Nitric Acid and H2O2 Solution was added to the Dried tissue.
- After 1 hour, the beaker was covered and kept for overnight.
- After overnight Nitric acid- H2O2 treatment, the tissue sample was homogenized to make a smooth suspension.
- Heat was applied to evaporate the solvent and heat dry the sample.
- The distilled water was added to dissolve the dried residuals and then collected in 15ml centrifuge tubes.

- These homogeneous suspensions were used to quantify arsenic deposition in gut samples by using Atomic Absorption Spectroscopy (Model number: FI-HG-AAS-AA 140 AAS-USA).

2) Recovery and Purification of Bacterial Strains Resistant to Arsenic:

Using strict aseptic techniques to prevent contamination, various segments of the gastrointestinal tract were sampled and placed into four Eppendorf tubes containing 1X phosphate-buffered saline (PBS). These gut samples were manually homogenized to produce a consistent bacterial suspension. This suspension was prepared in a total volume of 1ml by mixing 250 µl each from the duodenum, jejunum, ileum, and caecum samples.

Following the above method, samples were then diluted serially using sterile PBS. Identical volumes from each dilution were spread on nutrient agar (NA) plates to allow the growth of bacterial colonies during the incubation period. Plates with clearly countable colonies were selected for further microbiological investigation.

In addition to estimating the total culturable bacterial load, bacteria exhibiting resistance to arsenic (As) were selectively isolated from both control and arsenic-exposed groups. This was achieved by growing the bacterial suspensions on Mueller-Hinton Agar (MHA) containing 1 mM sodium arsenite ($NaAsO_2$), using the procedure outlined by Banerjee et al. (2011). The use of this arsenic-enriched medium applied selective pressure, enabling the isolation of arsenic-tolerant strains from each experimental group.

Colonies differing in morphological characteristics—such as color, texture, size, and shape—were selected for further purification. Pure bacterial isolates were obtained through the streak plate method, which separates individual strains from mixed cultures. Following incubation, distinct colonies were collected and preserved for future use in two forms: stab cultures at room temperature and glycerol stocks at -20°C, ensuring their long-term viability and availability for analysis.

3) Biochemical and Molecular Characterization of Arsenic-Resistant Bacterial Strains:

➢ Biochemical Characterization

Biochemical tests serve as a fundamental approach in microbiology used for identifying and distinguishing bacterial isolates by analyzing their specific metabolic functions. These assays evaluate bacterial enzyme mechanisms, leading to detetable outcomes like gas formation, color changes, or other reactions that reveal the presence or absence of particular enzymes. Such observable result of the enzymatic activities help to detect and differentiate several bacterial species.

Some of the most commonly employed biochemical assays include the catalase test, oxidase test, indole test, and various carbohydrate fermentation tests. Performing these tests helps generate a distinctive biochemical profile for each isolate. This profile can then be matched with established databases or reference materials to accurately identify the bacterial species.

Biochemical characterization is crucial in several areas of microbiology, including clinical diagnostics, environmental studies, microbial taxonomy, and industrial applications. By understanding the enzymatic and metabolic capabilities of antibiotic-resistant bacterial strains, researchers gain deeper insights into their identity, behavior, and potential impact, aiding in targeted interventions and further analysis.

The following Biochemical Tests were conducted:

1. Catalase Test

- **Principle:** To find out the presence of the catalase enzyme in the bacterial culture which catalyzes the hydrogen peroxide (H_2O_2)into O_2 and H_2O
- **Reagents/Media Composition:** Hydrogen peroxide solution (3%).
- **Procedure:** A small amount of colony growth from an overnight culture was placed on a glass slide using a sterile loop and a drop of 3% H_2O_2 was added to the culture over the slide
- **Positive Result:** Instant bubble formation with fizzing due to oxygen release
- **Negative Result:** No bubbling or fizzing

2. Oxidase Test

- **Principle:** Identifies the presence of cytochrome oxidase which catalyses the transport of electrons in electron transport chain, in overnight bacterial culture, which oxidizes the reagent
- **Reagents/Media Composition:** Oxidase reagent disks (tetramethyl-p-phenylenediamine dihydrochloride)(DD 018, Himedia).
- **Procedure:** Single colony from an overnight culture of NA medium (pH 7) was placed onto oxidase test disks, which provide electron donors for detecting cytochrome c oxidase activity.
- **Positive Result:** Development of a deep blue/purple colour within 10-30econds.
- **Negative Result:** No color change to purple/blue

3. Indole Test

- **Principle:** Detects the ability of bacteria to decompose tryptophan to indole
- **Reagents/Media Composition:** Tryptone broth: (5 g/L NaCl, 10 g/L tryptone), and Kovács reagent: (150 ml isoamyl alcohol, 10 g DMAB, 50 ml conc. HCl).
- **Procedure:** Single isolated colonies from NA plates were incoculated using sterile loop into 4ml tryptone medium and incubated for 24-48 hours at 37ºC. After this, 0.5 ml Kovac's reagent was added to aliquots of tryptone broth culture.
- **Positive Result:** Formation of a distinct cherry-red or pink layer on top of the medium within the reagent phase.
- **Negative Result:** No visible color change or yellowish layer after reagent addition.

4. Gelatin Hydrolysis Test

- **Principle:** Detects the bacteria's ability to synthesize gelatianse, that liquefies gelatin
- **Reagents/Media Composition:** An enzymatic digest of gelatin or peptone (5 g), beef extract (3 g), and gelatin (120 g) per 1lt. (pH 6.8).

- **Procedure:** A small bacterial inoculum was collected with an inoculating loop and stabbed into the tube containing gelatin medium. Both the inoculated and control tubes were kept in the incubator at 35°C for 48 hours. Following this, the tubes were refrigerated for 30 minutes to detect any liquefaction of the medium
- **Positive Result:** Liquefaction of the medium (gelatin) even after refrigeration
- **Negative Result:** Medium remains solid after refrigeration.

5. Methyl Red (MR) Test

- **Principle:** Tests the ability of bacterial culture to undergo mixed aid fermentation of glucose, resulting in stable acid production.
- **Reagents/Media Composition:** MR-VP broth: glucose (5 g/L), Peptone (7 g/L), and dipotassium phosphate (5 g/L). Methyl red indicator: 0.1% methyl red dissolved in 300 ml of 95% ethanol, followed by the addition of 200 ml of deionized water.
- **Procedure:** Tube containing 5ml MR-VP broth was inoculated with a sterile loop full of bacteria, and kept for 48 hours of incubation. After incubation, 2.5 ml culture broth was separated as an aliquot tube and 5 drops of methyl red indicator was added to detect color change
- **Positive Result:** Formation of red color (pH < 4.4). Examples: *E. coli*, *Yersinia sp.*, etc.
- **Negative Result:** Development of yellow or orange color (pH > 6.0). Examples: *Enterobacter aerogenes.*

6. Voges-Proskauer (VP) Test

- **Principle:** To test the production of acetoin by bacterial culture, a neutral end product of glucose fermentation
- **Reagents/Media Composition:** The MR-VP broth: glucose (5 g/L), peptone (7 g/L) and dipotassium phosphate (5 g/L). Barritt's Reagent A : 5% (w/v) α-naphthol disolved in absolute ethanol, while Barritt's Reagent B contains 40% (w/v) potassium hydroxide (KOH) in freshly prepared deionized water.
- **Procedure:** The left out 2.5 ml culture from the MR-VP broth was aliquoted into a separated tube and 0.6 ml Barritt's reagent A and 0.2 ml Barritt's reagent B were added to the the aliquot. After the addition of reagents, the tube was shaken for 30seconds to 1 minute and then left undisturbed for a minimum of 30 minutes. Exposure to atmospheric oxygen is essential to oxidize acetoin and produce the color change needed for the test.
- **Positive Result:** Appearance of red color within 30 minutes. Example: *Enterobacter aerogenes, Proteus mirabilis.*
- **Negative Result:** No color change or a copper hue. Example: *E. Coli, Shigella sp.*

7. Citrate Utilization Test

- **Principle:** Tests the ability of the bacterial culture to use citrate as its sole carbon source.
- **Reagents/Media Composition:** Simmon's Citrate Agar: (Sodium chloride (5.0g/ml), Sodium citrate (dehydrated- 2.0g/ml), Dipotassium phosphate (1.0g/ml), Ammonium dihydrogen phosphate (1.0g/ml), Magnesium sulphate (hepta hydrate – 0.2g/ml), Bromothymol blue (0.08g/ml), agar (15.0g/ml), etc.).

- **Procedure:** With a sterile inoculating loop, small bacterial culture was taken out and gently streaked onto the surface of the citrate agar slant. The tube was left loosely capped for incubation at 37°C for up to 7 days. Throughout this period of 7 days color change was observed .
- **Positive Result:** The presence of growth, with or without a color change from green to blue(alakaline pH) signifies the organism's ability to utilize citrate. Examples: *Salmonella, Citrobacter, Klebsiella, Enterobacter, Serratia,* etc.
- **Negative Result:** Lack of growth and no color change of medium from green. Examples: *E coli, Shigella, Morganella, Yersinia* etc.

▪ Gram Staining

Principle:
A differential staining technique used to classify bacteria into Gram-positive and Gram-negative, based on their cell wall organisation. Gram-positive bacteria with a thick peptidoglycan layer hold onto the primary stain (crystal violet), while Gram-negative bacteria having a thin peptidoglycan layer and an outer membrane, does not retain the crystal violet stain but takes up the counterstain (safranin).

Reagents/ Media Composition:

- **Primary stain:** Crystal violet (1% aqueous solution)
- **Mordant:** Iodine solution (1% aqueous solution)
- **Decolorizing agent:** 95% ethanol or acetone-alcohol mixture
- **Counterstain:** Safranin (0.25% aqueous solution)

Procedure:

1. A smear was made on a sterile glass slide with a loop full of inoculum on a drop of water and then heat-fixed by passing 2-3 times through flame.
2. After heat fixation, the primary stain- crystal violet was added dropwise and kept for 20 seconds, followed by a gentle wash with distilled water.
3. Then, the mordant- the Gram's Iiodine was added and let stand for 1 minute to create a dye-mordant complex and then again rinsed with water.
4. The smear was decolorized by applying ethanol or an acetone-alcohol solution for 10 to 30 seconds and rinsed again.
5. Finally, the slide was stained with the counter-stain, safranin and kept for one minute, rinsed gently with distilled water, and allowed to air dry.

Interpretation:

- **Gram-positive bacteria:** They appear purple due to the retention of the crystal violet stain. Examples: *Bacillus, Streptococcus, Staphylococcus, Enterococcus, Listeria, Lactobacillus, Nocardia, Streptomyces*, etc.
- **Gram-negative bacteria:** They appear pink or red due to loss of the primary stain after decolorisation but holding onto the safranin counterstain. Examples: *Escherichia coli, Salmonella, Shigella, Pseudomonas, Helicobacter,* etc.

➢ Molecular Characterization

- **Bacterial Genomic DNA Isolation:**

Tst tubes containing 5 ml Luria Broth were inoculated with well-isolated bacterial colonies and kept for incubation at 37°C for 24 hours. The HiPurA® Bacterial Genomic DNA Purification Kit was used to isolate genomic DNA from overnight bacterial culture. This kit employs a silica membrane-based spin column method, allowing for a streamlined workflow and the simultaneous processing of several samples.

The extraction process involves the following key steps:

1. Binding of DNA to the silica membrane within the spin column.

2. Removal of contaminants through a series of wash steps.

3. Elution of purified genomic DNA from the membrane.

After extraction, the quantity and quality of DNA were analysed by a spectrophotometer, and the DNA's integrity was confirmed by running it through a 0.8% agarose gel electrophoresis.

- **16S rRNA Gene Amplification by PCR:**

The isolated genomic DNA was used as the template for the amplification of the 16S rRNA gene through polymerase chain reaction (PCR), with each reaction carried out in a final volume of 50 µl, consisting of the following components:

1. Forward primer: 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) - 2 µl
2. Reverse primer: 1492R (5′-GGTTACCTTGTTACGACT T -3′) - 2 µl
3. DNA template (20 ng) - 1 µl
4. Molecular grade water - 20µl
5. PCR master mix - 25µl.

The master mix is composed of :

- DNA polymerase: Enzyme to synthesize new strands of DNA.
- dNTPs (deoxynucleotide triphosphates): Building blocks of new DNA strands.
- MgCl 2 : A molecule essential for enabling DNA polymerase to function properly.
- Buffer: This maintains the optimal pH and salt conditions for the PCR.

The PCR setup was –

Step	Temperature	Timing
Initial denaturation	94°C	4 minutes
Denaturation	94°C	1 minute
Annealing	55°C	1 minute
Extension	72°C	2 minutes
Final extension	72°C	10 minutes
Soak/ Infite hold	4°C	30 Cycles
Lid temperature	104°C	

Table 3: PCR Setup and required Temperature and Timings

The PCR products were observed by running through 1% agarose gel electrophoresis. Purification of the amplified DNA fragments was performed using the Himedia gel purification kit, and sequencing was outsourced by Nalgen Biotech.

4) Determination of MAR Index Using Antibiotic-Susceptibility test:

Determination of Antibiogram and MAR index of the strains:

Antibiotic susceptibility test: It was assessed using the disc agar diffusion technique using commercially procured antibiotic discs (HiMedia®, India). The selected antibiotics were:

Amikacin (30 µg), Aztreonam (30 µg), Cefazolin (30 µg), Cefoxitin (30 µg) , Cephalothin (30 µg), Cotrimoxazole (25 µg), Gentamicin (10 µg), Erythromycin (15 µg) , Oxacillin (1 µg), Ampicillin (10 µg) , Cefuroxime(30 µg), Ceftazidime (30 µg), Vancomycin (30 µg), Cefixime (5 µg), Cefotaxime (30 µg), Ceftriaxone(30 µg), Cefoperazone (75 µg), Cefepime (30 µg), Tetracycline (30 µg), Netillin (30 µg), Norfloxacin (10 µg), Levofloxacin (5 µg) , Polymyxin B (300 unit), Colistin (10 µg), Clindamycin (2 µg), Ciprofloxacin (5 µg).

Sl. No.	**Antibiotic Name**	**Generation**	**Class/ Category**	**Mode of action**
1	Gentamicin (GEN)	2	Aminoglycosides	Attach to the 30S ribosomal subunit inhibiting protein manufacture.
2	Amikacin (AK)	3		
3	Netillin (NET)	3		
4	Vancomycin (VA)	1	Glycopeptides	Attach to the D-Ala-D-Ala terminal preventing the formation of cell walls.
5	Oxacillin (OX)	2	Penicillin	Inhibiting bacterial cell wall formation by binding to penicillin-binding proteins (PBPs).
6	Ampicillin (AMP)	3		

7	Cefazolin (CZ)	1	Cephalosporin	Disrupt bacterial cell wall by binding to PBPs.
8	Cephalothin (CEP)	1		
9	Cefoxitin (CX)	2		
10	Cefuroxime (CXM)	2		
11	Ceftazidime (CAZ)	3		
12	Cefixime (CFM)	3		
13	Cefotaxime (CTX)	3		
14	Ceftriaxone (CTR)	3		
15	Cefoperazone (CPZ)	3		
16	Cefepime (CPM)	4		
17	Aztreonam (AT)	1	Monobactam	Disrupts cell wall formation by hindering the synthesis of peptidoglycan
18	Tetracycline (TE)	1	Tetracyclin	Block bacterial protein synthesis by attaching to the 30S ribosome subunit
19	Norfloxacin (NX)	1	Quinolones/ Fluoroquinolones	Disrupt bacterial DNA replication by interfering with the DNA gyrase and topoisomerase IV mechanisms
20	Ciprofloxacin (CIP)	2		
21	Levofloxacin (LE)	3		
22	Erythromycin (E)	1	Macrolides	Block bacterial protein synthesis by attaching to the 50S ribosomal subunit
23	Cotrimoxazole (COT)	__	Sulphonamides	Inhibit the bacterial folic acid synthesis
24	Polymyxin B (PB)	1	Polymyxins	Disrupt bacterial cell membranes by interacting with phospholipids
25	Colistin (CL)	1		
26	Clindamycin (CD)	2	Lincosamides	Block bacterial protein synthesis by attaching to the 50S ribosomal subunit
27	Chloramphenicol (C)	__	Amphenicols	Block bacterial protein synthesis by attaching to the 50S ribosomal subunit

Table 4: Summary of the selected 27 Antibiotics

- **Experiments Performed:**

  - In addition to isolating the total culturable gut microbiota, arsenic (As)-resistant bacterial strains were selectively obtained from various groups of experimental animals by applying selection pressure on Mueller-Hinton Agar (MHA) plates. These arsenic-tolerant bacteria were further purified using the Streak Plate technique as described by Duranti et al. (2017).
  - To obtain liquid cultures, 5 ml of Mueller-Hinton Broth (MHB) medium was inoculated with single, well-isolated colonies from MHA plates with and allows to incubate in a shaker at 37°C with shaking at 120±10 rpm for 12 hours. After incubation, the bacterial cell density of each culture was assessed spectrophotometrically at 660 nm.
  - Based on the calculated cell density, a 1 McFarland standard bacterial suspension was prepared and uniformly spread on fresh MHA plates. A total of 27 different Gibco™ antibiotic discs were carefully placed on the agar surface. The plates were allowed to incubate at 37°C for 24 hours, after which the zones of inhibition around each disc were measured to determine bacterial susceptibility.
  - Each bacterial isolate's response to the antibiotics was classified as sensitive, moderately sensitive, or resistant based on the diameter of the observed inhibition zones, as per the manufacturer's interpretive guidelines, which align with the National Committee for Clinical Laboratory Standards (NCCLS) criteria (Maden et al., 2011; Shakya et al., 2012).
  - The isolates were further determined for the MAR Index values that demonstrated resistance to antibiotics, using the standardized interpretive table recommended by the NCCLS (Thakurta et al., 2007).

RESULTS

- **Record of Arsenic concentration in rat gut sample :**

The concentration of arsenic in gut samples of some of the model animals (n=3) of control and treated groups was quantified using atomic absorption spectroscopy (FI-HG-AAS- AA 140 AAS, USA) according to the standard measurement protocol.

Model		Rat Code	OD	Concentration (µg/L)	Initial Weight (gm)	Conversion Factor	Final Volume Taken	Concentration of Arsenic (ppb)	Mean Arsenic concentration of each group (ppb)
Female	Female Control	AF1	0.0697	3.94	0.203	1.5	4	116.45	
		AF2	0.0505	2.87	0.199	1.5	4	86.53	
		AF3	0.0873	4.91	0.203	1.5	4	145.12	116.04±29.297
	Female NaAsO2 treated	BF1	0.2279	12.79	0.205	1.5	4	374.34	
		BF2	0.3358	19.2	0.21	1.5	4	548.57	
		BF3	0.3166	17.89	0.203	1.5	4	528.77	483.89±95.390
Male	Male Control	AM1	0.0734	4.01	0.203	1.5	4	118.52	
		AM2	0.0514	2.92	0.201	1.5	4	87.16	
		AM3	0.0816	4.6	0.209	1.5	4	132.06	112.58±23.029
	Male NaAsO2 treated	BM1	0.0912	5.12	0.207	1.5	4	148.41	
		BM2	0.3056	17.74	0.208	1.5	4	511.73	
		BM3	0.3228	18.31	0.209	1.5	4	525.65	395.26±213.896

Table 5: Record of Arsenic concentration in gut samples of different groups of rats

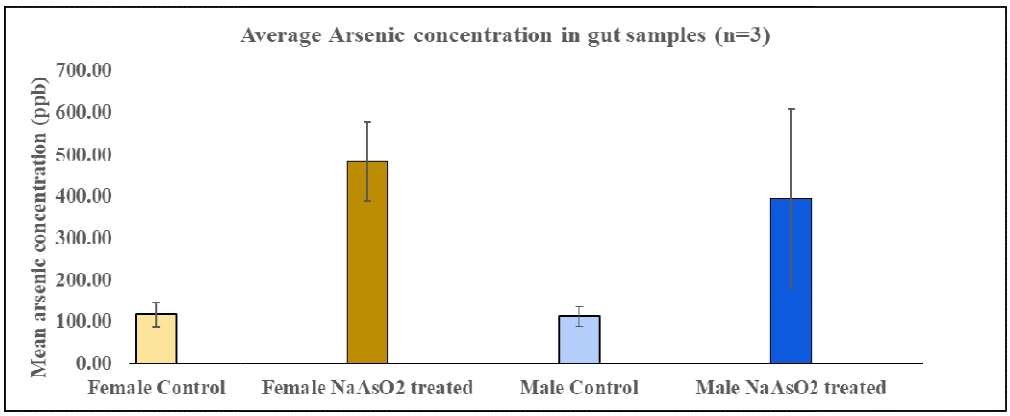


Fig 3: The mean Arsenic concentration in gut samples of different groups and sexes of rats

Arsenic concentration in the rat gut sample of the treated group of model animals is significantly much higher than the control group of model animals for both male and female Wistar rats (Fig 3). So, we can conclude that the orally administered NaAsO2 is successfully assimilated in the body of the rat of the treated group.

- **Isolation and purification of As-tolerant bacterial strains:**
  From a total of 24 Wistar rat gut samples, we have successfully isolated and purified 59 different bacterial strains based on colony morphology, basic microscopic

observation, and some biochemical tests. From control and As-treated groups, we have isolated 33 and 26 bacterial isolates respectively.

Test	Control Positive	Control Negative	Treated Positive	Treated Negative
Catalase Test	33	0	26	0
Oxidase Test	0	33	23	3
Gelatin-Hydrolysis Test	8	25	4	22
Indole Test	5	21	5	21
Citrate Utilisation Test	2	31	5	21
MR Test	9	24	6	20
VP Test	3	30	4	22

Gram Staining	Control Gram Positive	Control Gram Negative	Treated Gram Positive	Treated Gram Negative
	26	7	17	9

Table 6: Numbers of strains positive or negative to the performed biochemical tests

- **Differential abundance of the average viable and As-tolerant bacterial strains:**

  The below-mentioned table (Table 6) shows the differential abundance of the average viable and As-tolerant bacterial strains in gut samples of different model animals of each group in terms of Colony Forming Unit (CFU) per ml. Here, due to the addition of selection pressure, colony count is decreasing in gut samples of each model animal group.

Column 1	Column 2	Average Colony Count in Enrichment Media (CFU/ml)	Average Colony Count in (Enrichment Media + 1mM As) (CFU/ml)
Male	Male Control	784750	44950
	Male As-treated	489166.6667	277833.3333
Female	Female Control	343500	25333.33333
	Female As-treated	296100	142416.6667

Table 7: Differential abundance of the average viable and As-tolerant bacterial strains in gut samples of different groups of model animals

If we only consider the 2 main groups of model animals irrespective of their male and female individual, the following data will be obtained (Table 7). In both groups, there was a reduction in number of CFU in the presence of Arsenic selection pressure.

Animal Group	Total Colony Count in MHA without As (CFU/ml)	Total Colony Count in MHA with 1Mm As (CFU/ml)	Percentage of total CFU showing resistance to As (%)
Control group	6769500	421700	6.23
As-treated group	4711600	2521500	53.52

Table 8: Differential abundance of total viable and As-tolerant bacterial strains in the Control and As-treated group and respective percentages of Resitance to As

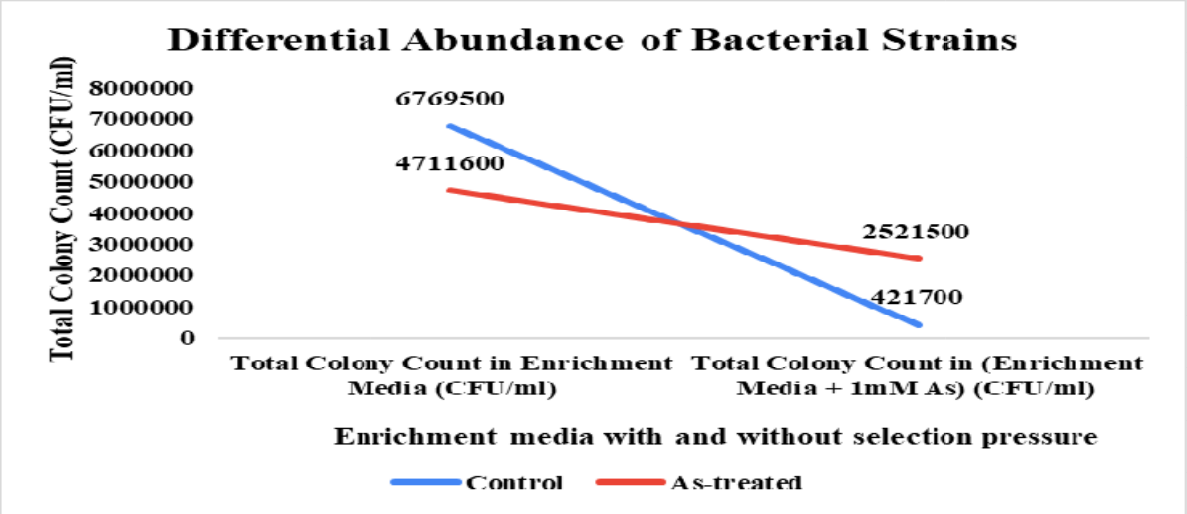


Fig 4: Differential abundance of total colony count in Enrichment media and As-selection pressure of Control and As-treated group

The Control group shows a **93.77%** reduction in bacterial population when exposed to 1Mm As, indicating that most strains were not arsenic-resistant**.** While the As-treated group experiences only a **46.48%** reduction, suggesting partial adaptation or selection of arsenic-resistant bacterial strains.

- **Results of Antibiotic Susceptibility test:**

Determination of antibiogram was performed by antibiotic susceptibility test. After culturing the purified, well-isolated colonies in 5 ml of Mueller-Hinton Broth, the cell density of each suspension was assessed using a spectrophotometric technique at 660 nm. Using the measured optical density, a bacterial suspension adjusted to match the 1 McFarland standard was uniformly spread over Mueller-Hinton Agar (MHA) plates. Gibco™ antibiotic discs were then placed onto the surface, and after incubating the plates at 37°C for 24 hours, the diameters of the resulting inhibition zones were measured. The bacterial isolates were classified as sensitive, intermediate, or resistant to specific antibiotics by comparing the measured inhibition zone diameters with the interpretive standards provided by the manufacturer, which followed the NCCLS recommendations (Maden et al., 2011; Shakya et al., 2012).

Group	Strain No.	Number of Antibiotics to which a particular strain is showing			MAR index	Mean MAR Index
		Sensitive (S)	Moderately sensitive (MS)	Resistant (R)		
Control	1	18	6	3	0.1111	0.0763
	2	17	8	2	0.0741	
	3	12	11	4	0.1481	
	4	15	9	3	0.1111	
	5	15	8	4	0.1481	
	6	15	11	1	0.0370	
	7	19	5	3	0.1111	
	8	20	7	0	0.0000	
	9	18	8	1	0.0370	
	10	20	7	0	0.0000	
	11	19	6	2	0.0741	
	12	21	5	1	0.0370	
	13	22	4	1	0.0370	
	14	26	0	1	0.0370	
	15	23	3	1	0.0370	
	16	24	0	3	0.1111	
	17	25	1	1	0.0370	
	18	24	1	2	0.0741	
	19	24	1	2	0.0741	
	20	21	4	2	0.0741	
	21	23	3	1	0.0370	
	22	23	2	2	0.0741	
	23	22	1	4	0.1481	
	24	20	4	3	0.1111	
	25	24	2	1	0.0370	
	26	23	2	2	0.0741	
	27	25	1	1	0.0370	
	28	22	3	2	0.0741	
	29	22	2	3	0.1111	
	30	24	1	2	0.0741	
	31	22	1	4	0.1481	
	32	24	0	3	0.1111	
	33	21	3	3	0.1111	
As-treated	1	9	6	12	0.4444	
	2	6	5	16	0.5926	
	3	16	5	6	0.2222	
	4	11	3	13	0.4815	
	5	9	4	14	0.5185	

	6	15	2	10	0.3704	
	7	16	3	8	0.2963	
	8	14	1	12	0.4444	
	9	21	3	3	0.1111	
	10	15	4	8	0.2963	
	11	19	4	4	0.1481	
	12	21	4	2	0.0741	
	13	19	5	3	0.1111	
	14	23	3	1	0.0370	0.2108
	15	20	4	3	0.1111	
	16	14	3	10	0.3704	
	17	22	4	1	0.0370	
	18	20	6	1	0.0370	
	19	21	5	1	0.0370	
	20	23	3	1	0.0370	
	21	17	7	3	0.1111	
	22	23	3	1	0.0370	
	23	17	3	7	0.2593	
	24	18	6	3	0.1111	
	25	17	7	3	0.1111	
	26	23	2	2	0.0741	

Table 9: Antibiotic susceptibility test outcomes for each bacterial isolate from both the control and arsenic-treated groups.

	The average number of antibiotics of each group for:		
Group of animals	Sensitive	Moderately sensitive	Resistant
Control	21.00	3.94	2.06
As-Treated	17.27	4.04	5.59

Table 10: Summary of the Antibiotic susceptibility test outcomes for each bacterial isolate from both the control and arsenic-treated groups

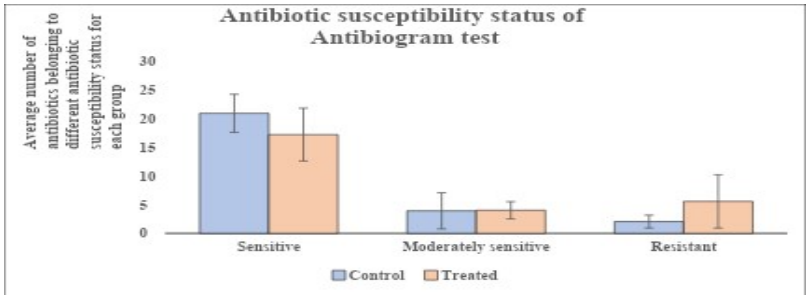


Fig 5: Graphical representation of the results of the Antibiotic Susceptibility Test of Control and As-treated groups

Table 8 and Table 9 depict that the average number of antibiotics belonging to the Sensitive category is decreasing in the As-treated group as against the Control group. Figure 6 is further validating the above result. The average number of antibiotics belonging to the

Resistant group is increasing in the As-treated group as compared to the Control group. The acquired data (Figure 6) depicts that, arsenic exposure might have some impacts on the antibiotic resistance pattern of mammalian gut prokaryotes.

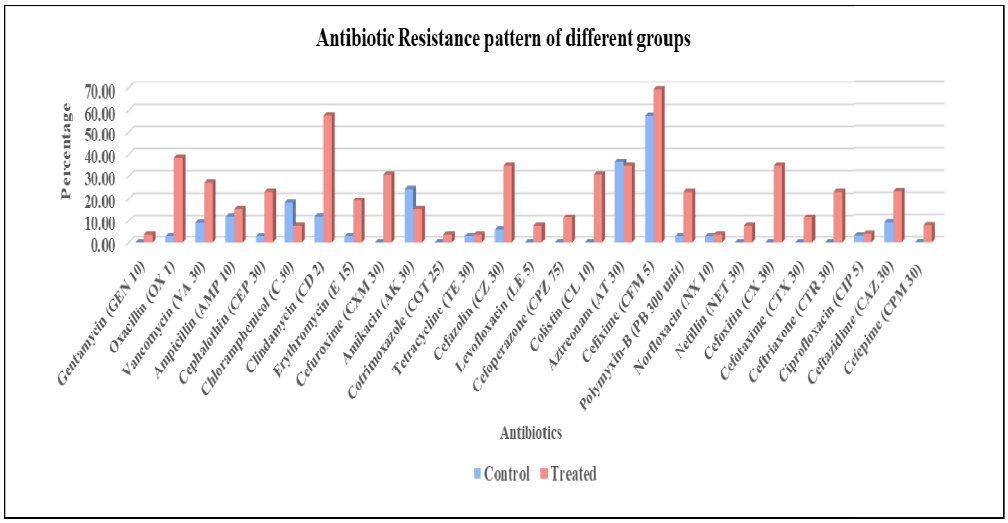


Fig 6: Comparative study of Antibiotic Resistance pattern in between Control and As-treated groups

Bacterial isolates from the treated group were resistant to all the 27 antibiotics tested, but the control group isolates were resistant to only 16 (Figure 6), indicating a broader resistance spectrum. The MAR Index of those enumerated antibiotic-resistant bacteria was calculated from the acquired data with the help of the interpretive table that is recommended by NCCLS (Thakurta et al., 2007).

Group (n= 59)	MAR Index
Control (n= 33)	0.076±0.04
As-treated (n=26)	0.211±0.17

Table 11: Calculated MAR Index of Control and As-treated groups

The observed calculations in Table 10 provide an insight that the MAR index in the treated group was approximately three times higher, suggesting that arsenic exposure co-regualtes antibiotic resistance in gut bacterial strains, which is illustrated in Figure 7. Such results highlight arsenic's role as a selective pressure in promoting multidrug resistance.

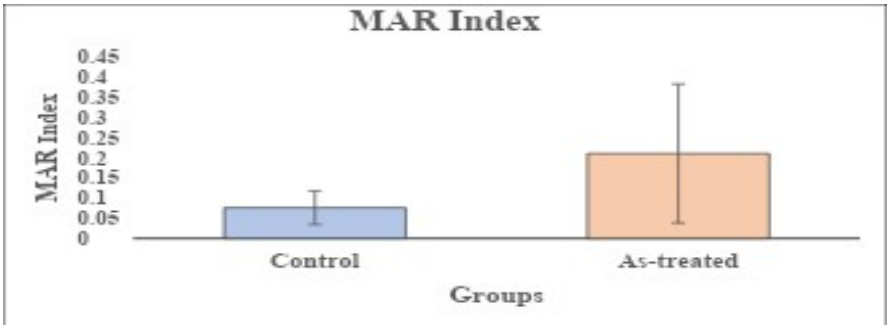


Fig 7: Graphical representation of calculated MAR Index of Control and As-treated groups

SUPPLEMENTARY DATA

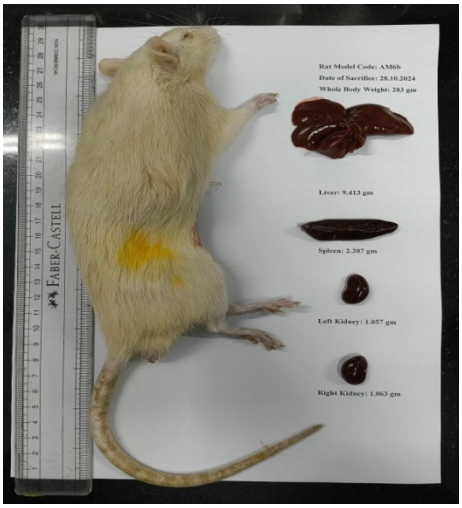


Fig 8: Control Wistar Rat model sacrifice showing its height and separated organs for calculating Mean Organ Index

Fig 9: Arsenic-Treated Wistar Rat model sacrifice howing its height and separated organs for calculating Mean Organ Index

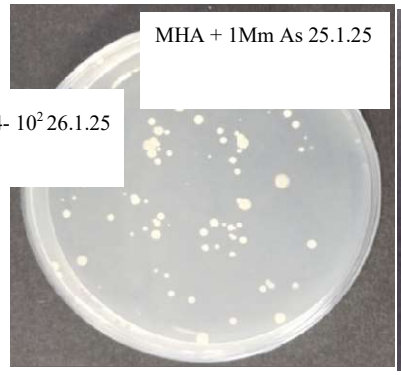


Fig 10: Isolation of arsenic-tolerant bacterial strains

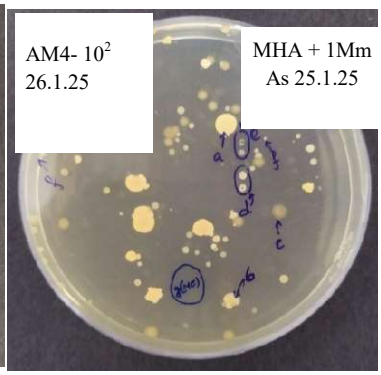


Fig 11: Characterization of arsenic-tolerant bacterial strains in terms of colony morphology

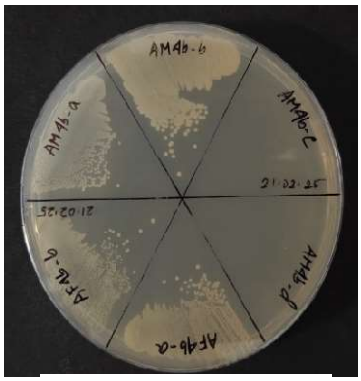
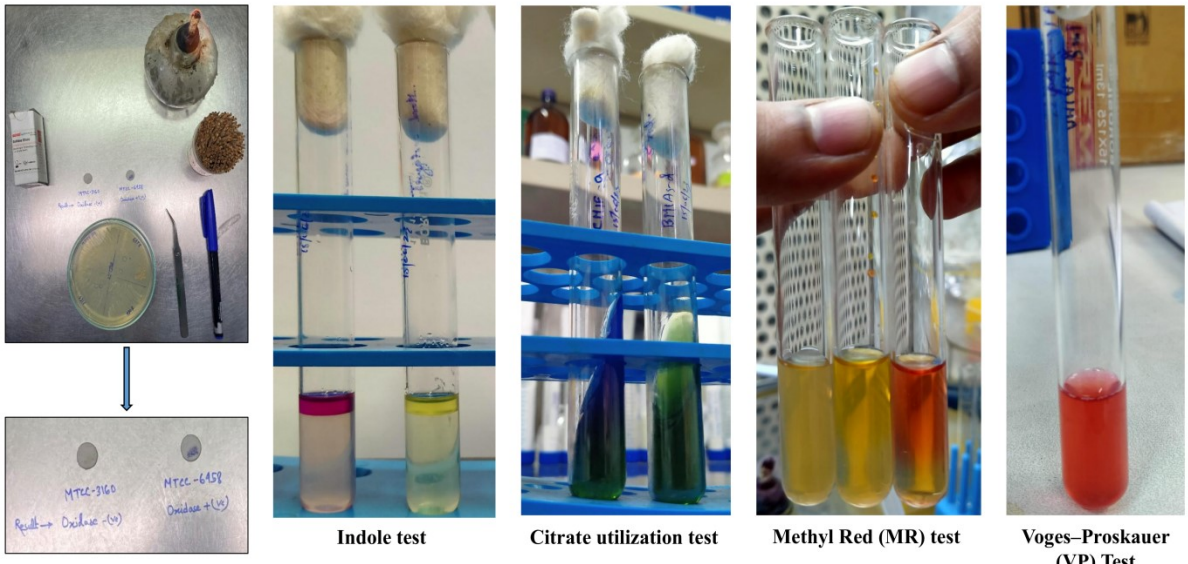


Fig 12: Purification of arsenic-tolerant bacterial strains by Streak plate



Oxidase test | Indole test | Citrate utilization test | Methyl Red (MR) test | Voges–Proskauer (VP) Test

Fig 13: Results of Different Biochemical Tests conducted for the Isolated Bacterial Strains

Gram positive Cocci | Gram positive Small Rod

Gram Staining

Gram negative Bacillus | Gram negative Small Rod

Fig 14: Results of Conducted Gram Staining for the Isolated Bacterial Strains

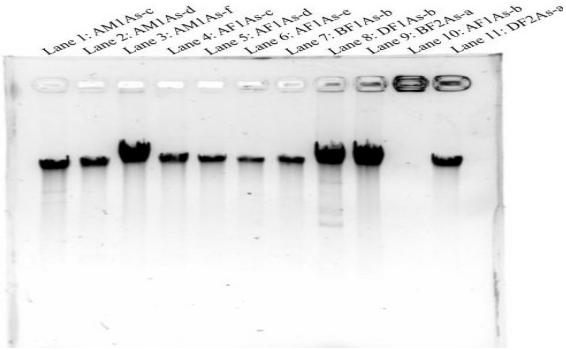


Fig 15: Molecular Identification by Isolation of Genomic DNA from different Isolated Bacterial Strains

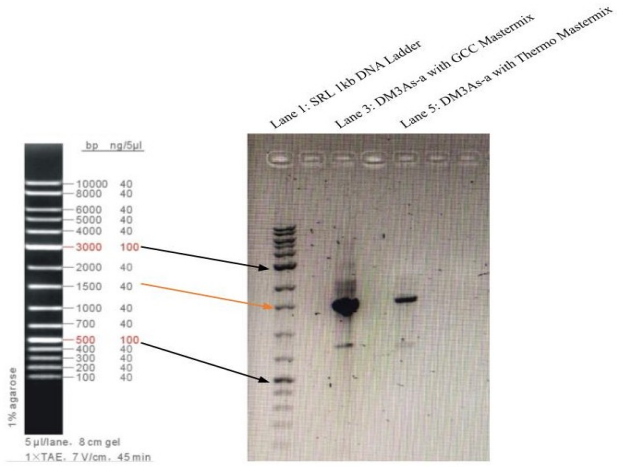


Fig 16: Result of Polymerase Chain Reaction (PCR) of Iolated Bacterial Genomic DNA

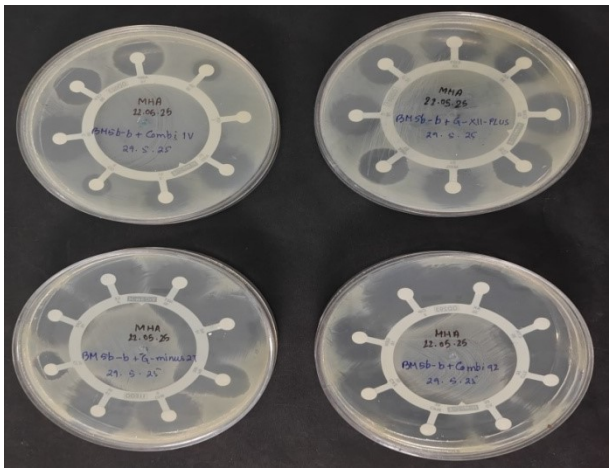


Fig 17: Determination of sensitivity of the arsenic-resistant bacteria for different antibiotics by the standard disc-agar diffusion method on Mueller-Hinton Agar (MHA) plates

Fig 18 : Selected Choice of Antibiotic combinations

CONCLUSION

This research study was directed to isolate and characterize bacterial strains particularly resistant to arsenic from mammalian gut using Wistar rats as model animal. Prolonged subchronic exposure to sodium arsenite during the study resulted in modifications of gut microbiota organization and an increased presence of bacteria exhibiting resistance to both arsenic and multiple antibiotics. These findings highlight the growing concern over environmental contaminants and the escalating issue of antibiotic resistance, both of which represent significant public health risks.

Gut microbes are crucial for maintaining general well-being by facilitating digestion, modulating immune responses, and producing essential metabolic substances. However, environmental toxins such as arsenic can disrupt this delicate microbial equilibrium, leading to a condition known as dysbiosis. The findings of the study indicated that arsenic exposure created selective pressure that favored the survival and growth of bacteria resistant to both arsenic and antibiotics in the exposed group, in comparison to control. This shift resulted in a reduction of beneficial commensal bacteria, thereby impairing normal gut functions.

A total of 59 arsenic-resistant bacterial isolates from the rat gut were characterized for their biochemical and molecular characteristics. When Antibiotic-susceptibility tests were carried out, the results showed the treated group was resistant to all the 27 chosen antibiotics while the strains from the control group were found to be resistant only against 16 antibiotics. This sharp contrast indicates that arsenic exposure is not only toxic to the gut environment but also promotes the selection of MAR traits in the resident bacteria. The MAR index for the arsenic-treated group was found to be three times higher than the control group, underlining a clear correlation between heavy metal exposure and the rise of antibiotic-resistant genes.

Atomic Absorption Spectroscopy (AAS) results confirmed the accumulation of arsenic in the gut, validating the biological exposure model. This exposure highlights the increased evidence of physiological and microbial changes in the potential arsenic-contaminated regions of India and Bangladesh especially. The isolated and examined bacterial strains can thus serve as biological indicators of arsenic contamination and can be explored for future use in bioremediation purposes given their capacity to tolerate toxic metal levels.

Overall, this study culminates the dual threat posed by arsenic contamination in environments. Firstly, the delicate balance of gut microbiota is altered posing serious health disorders. Secondly, arsenic exposure contributes to decline of commensal species while escalating the growth of resistant species both to arsenic as well as several antibiotics causing a serious concern over antibiotic resistance. These results emphasize the need for immediate attention towards monitoring environmental contaminants and reassessing the uncontrolled application of heavy metals in both industrial and agricultural sectors.

Moving forward, research should aim to pinpoint the exact resistance genes involved and explore the prolonged effects of heavy metal-driven gut microbial imbalance on overall human health. Further experimental studies can explore the expressions of arsenic-resistant and antibiotic-resistant genes respectively due to the selection pressure of arsenic on treated groups. Knowledge can be gained from the studies of co-regulation between the 2 resistant gene types if present any. The knowledge gained from this investigation can play a valuable role in shaping regulatory policies and guiding public health strategies to reduce exposure to harmful environmental substances and curb the escalation of antibiotic resistance.

APPENDIX

Column 1	Column 2	Column 3	Total Colony Count in MHA without As	Total Colony Count in MHA with 1mM As	Average Colony Count in MHA without As	Average Colony Count in MHA with 1mM As	SD for Colony Count in MHA without As	SD for Colony Count in MHA with 1mM As	Total Colony Count in MHA without As	Total Colony Count in MHA with 1mM As
Male	Male Control	Rat 1	53500	33100	784750	44950	1202466.9	65590.26223	470850	269700
		Rat 2	53000	21600						
		Rat 3	72000	8200						
		Rat 4	240000	2700						
		Rat 5	3390000	190000						
		Rat 6	900000	14100						
	Male As-treated	Rat 1	1260000	920000	489166.6667	277833.3333	414340.6073	308442.0079	293500	166700
		Rat 2	302000	235000						
		Rat 3	113000	71000						
		Rat 4	770000	40000						
		Rat 5	70000	50000						
		Rat 6	420000	351000						
Female	Female Control	Rat 1	690000	27700	343500	25333.33333	237011.7789	11967.13091	206100	152000
		Rat 2	14000	2200						
		Rat 3	124000	28000						
		Rat 4	270000	20000						
		Rat 5	390000	35000						
		Rat 6	573000	39100						
					296100	142416.6667	280642.5663	189489.7131	177660	854500
	Female As-treated	Rat 1	32600	18900						
		Rat 2	203000	81600						
		Rat 3	850000	560000						
		Rat 4	20000	13700						
		Rat 5	400000	84000						
		Rat 6	271000	96300						

Table 12: Total, Average, and Standard Deviation of Bacterial Colony Counts on MHA Plates With and Without 1mM Arsenic (As) Exposure in Male and Female Rat Groups (Control vs. Treated)

REFERENCES

1. **Afzaal, M., Saeed, F., Shah, Y. A., Hussain, M., Rabail, R., Socol, C. T., ... & Aadil, R. M. (2022). Human gut microbiota in health and disease: Unveiling the relationship. *Frontiers in microbiology*, *13*, 999001.**
2. **AboNahas, H. H., Darwish, A. M., Abd EL-kareem, H. F., AboNahas, Y. H., Mansour, S. A., Korra, Y. H., ... & Saied, E. M. (2022). Trust your gut: the human gut microbiome in health and disease. *Microbiome-gut-brain Axis: Implications on health*, 53-96.**
3. **Giambò, F., Costa, C., Teodoro, M., & Fenga, C. (2022). Role-playing between environmental pollutants and human gut microbiota: A complex bidirectional interaction. *Frontiers in Medicine*, *9*, 810397.**
4. **Morgan, X. C., Tickle, T. L., Sokol, H., Gevers, D., Devaney, K. L., Ward, D. V., ... & Huttenhower, C. (2012). Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome biology*, *13*, 1-18.**
5. **Wang, X. Q., Zhang, A. H., Miao, J. H., Sun, H., Yan, G. L., Wu, F. F., & Wang, X. J. (2018). Gut microbiota as important modulator of metabolism in health and disease. *RSC advances*, *8*(74), 42380-42389.**
6. **Alonso, V. R., & Guarner, F. (2013). Linking the gut microbiota to human health. *British Journal of Nutrition*, *109*(S2), S21-S26.**
7. **Gomaa, E. Z. (2020). Human gut microbiota/microbiome in health and diseases: a review. *Antonie Van Leeuwenhoek*, *113*(12), 2019-2040.**
8. **Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., ... & Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the national academy of sciences*, *101*(44), 15718-15723.**
9. **Round, J. L., & Mazmanian, S. K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nature reviews immunology*, *9*(5), 313-323.**
10. **Woting, A., & Blaut, M. (2016). The intestinal microbiota in metabolic disease. *Nutrients*, *8*(4), 202.**
11. **Basson, A., Trotter, A., Rodriguez-Palacios, A., & Cominelli, F. (2016). Mucosal interactions between genetics, diet, and microbiome in inflammatory bowel disease. *Frontiers in immunology*, *7*, 290.**
12. **Hapfelmeier, S., Lawson, M. A., Slack, E., Kirundi, J. K., Stoel, M., Heikenwalder, M., ... & Macpherson, A. J. (2010). Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. *Science*, *328*(5986), 1705-1709.**
13. **Mestecky, J., Russell, M. W., & Elson, C. O. (1999). Intestinal IgA: novel views on its function in the defence of the largest mucosal surface. *Gut*, *44*(1), 2-5.**
14. **Brüssow, H. (2020). Problems with the concept of gut microbiota dysbiosis. *Microbial biotechnology*, *13*(2), 423-434.**
15. **Bischoff, S. C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J. D., Serino, M., ... & Wells, J. M. (2014). Intestinal permeability–a new target for disease prevention and therapy. *BMC gastroenterology*, *14*, 1-25.**
16. **Makki, K., Deehan, E. C., Walter, J., & Bäckhed, F. (2018). The impact of dietary fiber on gut microbiota in host health and disease. *Cell host & microbe*, *23*(6), 705-715.**
17. **Reddy, B. S., & Rivenson, A. (1993). Inhibitory effect of Bifidobacterium longum on colon, mammary, and liver carcinogenesis induced by 2-amino-3-methylimidazo [4, 5-f] quinoline, a food mutagen. *Cancer research*, *53*(17), 3914-3918.**
18. **Kinross, J. M., Darzi, A. W., & Nicholson, J. K. (2011). Gut microbiome-host interactions in health and disease. *Genome medicine*, *3*, 1-12.**
19. **Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., & Reddy, D. N. (2015). Role of the normal gut microbiota. *World journal of gastroenterology: WJG*, *21*(29), 8787.**
20. **Neuman, H., Forsythe, P., Uzan, A., Avni, O., & Koren, O. (2018). Antibiotics in early life: dysbiosis and the damage done. *FEMS microbiology reviews*, *42*(4), 489-499.**
21. **Francino, M. (2016). Antibiotics and the human gut microbiome: dysbioses and accumulation of resistances. *Frontiers in microbiology*, *6*, 164577.**
22. **Parkin, K., Christophersen, C. T., Verhasselt, V., Cooper, M. N., & Martino, D. (2021). Risk factors for gut dysbiosis in early life. *Microorganisms*, *9*(10), 2066.**
23. **Rodríguez-Lado, L., Sun, G., Berg, M., Zhang, Q., Xue, H., Zheng, Q., & Johnson, C. A. (2013). Groundwater arsenic contamination throughout China. *Science*, *341*(6148), 866-868.**
24. **Jin, Y., Wu, S., Zeng, Z., & Fu, Z. (2017). Effects of environmental pollutants on gut microbiota. *Environmental Pollution*, *222*, 1-9.**
25. **Arneth, B. M. (2018). Gut–brain axis biochemical signalling from the gastrointestinal tract to the central nervous system: gut dysbiosis and altered brain function. *Postgraduate medical journal*, *94*(1114), 446-452.**
26. **Crumeyrolle-Arias, M., Jaglin, M., Bruneau, A., Vancassel, S., Cardona, A., Daugé, V., ... & Rabot, S. (2014). Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology*, *42*, 207-217.**
27. **Zhang, Z., Wang, Y., & Li, Q. (2018). Mechanisms underlying the effects of stress on tumorigenesis and metastasis. *International Journal of Oncology*, *53*(6), 2332-2342.**
28. **Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain research*, *1693*, 128-133.**
29. **Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., & Sadeghi, M. (2021). Toxic mechanisms of five heavy metals: Mercury, lead, chromium, cadmium, and arsenic. *Frontiers in Pharmacology, 12*, 643972.**
30. **Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., ... & Burcelin, R. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes, 56*(7), 1761–1772.**
31. **Frank, D. N., St. Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N., & Pace, N. R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences, 104*(34), 13780–13785.**
32. **Guan, Q. (2019). A comprehensive review and update on the pathogenesis of inflammatory bowel disease. *Journal of Immunology Research*, 2019, 7247238.**

33. **Li, H., Wang, Y., Liu, X., Zhang, Y., Ma, D., & Li, X. (2022). Gut microbiota and aging: Implications for health and age-related diseases.** ***Journal of Translational Medicine, 20*****, 377.**
34. **Rosenfeld, C. S. (2017). Gut dysbiosis in animals due to environmental chemical exposures.** ***Frontiers in Cellular and Infection Microbiology, 7*****, 396.**
35. **Bajinka, O., Tan, Y., Abdelhalim, K. A., Özdemir, G., Qiu, S., & Bo, X. (2020). Extrinsic factors influencing gut microbes, the immediate consequences and restoring eubiosis.** ***AMB Express*****, 10(1), 130.**
36. **Cheng, Y., Jin, Y., Li, X., Li, Y., Zhang, Y., & Zhao, H. (2021). Titanium dioxide nanoparticles induced intestinal inflammation in mice via ROS-mediated TLR4 signaling pathway.** ***Environmental Toxicology and Pharmacology*****, 87, 103686.**
37. **Claus, S. P., Guillou, H., & Ellero-Simatos, S. (2016). The gut microbiota: A major player in the toxicity of environmental pollutants?** ***NPJ Biofilms and Microbiomes*****, 2, 16003.**
38. **Hadrup, N., Loeschner, K., Bergström, A., Wilcks, A., Gao, X., Vogel, U., ... & Lam, H. R. (2012). Subacute oral toxicity investigation of nano- and micro-sized copper oxide particles in rats.** ***Archives of Toxicology*****, 86(7), 1103–1112.**
39. **Joly Condette, C., Bach, V., Mayeur, C., Gay-Queheillard, J., & Khorsi-Cauet, H. (2015). Chlorpyrifos exposure during perinatal period affects intestinal microbiota associated with delay of maturation of digestive tract in rats.** ***Journal of Pediatric Gastroenterology and Nutrition*****, 61(1), 30–40.**
40. **Lai, K. P., Chung, Y. T., Li, J. W., Wan, H. T., Wong, C. K. C. (2016). Bisphenol A alters gut microbiome: Comparative metagenomics analysis.** ***Environmental Pollution*****, 218, 923–930.**
41. **Liu, J., Liu, Y., Shao, Y., Li, X., & Zhang, Y. (2016). Gut microbiota and endocrine disruptors: In search of mechanism of action.** ***Environmental Pollution*****, 208(Pt B), 543–551.**
42. **Mai, V., & Draganov, P. V. (2013). Recent advances and remaining gaps in understanding the interactions between the gut microbiota and infectious diarrheal diseases.** ***Therapeutic Advances in Gastroenterology*****, 6(1), 15–22.**
43. **Mesnage, R., & Antoniou, M. N. (2017). Facts and fallacies in the debate on glyphosate toxicity.** ***Frontiers in Public Health*****, 5, 316.**
44. **Modi, S. R., Collins, J. J., & Relman, D. A. (2014). Antibiotics and the gut microbiota.** ***Journal of Clinical Investigation*****, 124(10), 4212–4218.**
45. **National Institute of Environmental Health Sciences. (2021).** ***Bisphenol A (BPA)*****.**
46. **Rogers, G. B., & Aronoff, D. M. (2016). The influence of non-antibiotic drugs on the gut microbiota.** ***Current Opinion in Microbiology*****, 31, 45–50.**
47. **Song, Y., Li, L., Shao, L., Zhang, Y., Zhang, L., & Liu, Y. (2015). Microplastics in the environment: A review of their occurrence, fate, and effects.** ***Science of the Total Environment*****, 536, 1139–1149.**
48. **Syromyatnikov, M. Y., Yablokov, A. V., & Syromyatnikova, A. V. (2020). Glyphosate and its influence on the environment and human health: A review.** ***Environmental Science and Pollution Research*****, 27(16), 18320–18334.**
49. **Vanner, S. (2008). Antibiotics and the gut: Does antibiotic exposure lead to harmful changes in gut bacteria?** ***Canadian Journal of Gastroenterology*****, 22(7), 535–536.**
50. **Zhu, D., Bi, Q., Xiang, H., Zheng, Y., Tang, Y., & Meng, W. (2018). Microplastic exposure alters the gut microbiota and increases postnatal metabolic disorders in mice.** ***Environmental Pollution*****, 243(Pt A), 534–543.**
51. **Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. J. (2018). Environmental factors influencing the development and spread of antibiotic resistance.** ***FEMS microbiology reviews*****,** ***42*****(1), fux053.**
52. **Baker-Austin, C., Wright, M. S., Stepanauskas, R., & McArthur, J. V. (2006). Co-selection of antibiotic and metal resistance.** ***Trends in Microbiology*****, 14(4), 176–182.**
53. **Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. V. (2015). Molecular mechanisms of antibiotic resistance.** ***Nature Reviews Microbiology*****, 13(1), 42–51.**
54. **Chowdhury, S., et al. (2016). Environmental reservoirs of antibiotic resistance genes.** ***Environmental Science & Technology*****, 50(6), 3060–3070.**
55. **Cusumano, G., Angeles Flores, G., Venanzoni, R., & Angelini, P. (2025). The impact of antibiotic therapy on intestinal microbiota: Dysbiosis, antibiotic resistance, and restoration strategies.** ***Antibiotics*****, 14(4), 371.**
56. **De La Cochetière, M. F., Durand, T., Lepage, P., Bourreille, A., Galmiche, J. P., & Doré, J. (2005). Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge.** ***Journal of Clinical Microbiology*****, 43(11), 5588–5592.**
57. **Doucet-Populaire, F., Trieu-Cuot, P., Dosbaa, I., Andremont, A., & Courvalin, P. (1991). Inducible transfer of conjugative transposon Tn1545 from Enterococcus faecalis to Listeria monocytogenes in the digestive tracts of gnotobiotic mice.** ***Antimicrobial Agents and Chemotherapy*****, 35(1), 185–187.**
58. **Faleye, T. O. C., Adegoke, A. A., & Adeniji, J. A. (2019). Environmental reservoirs of antibiotic resistance genes.** ***Journal of Global Antimicrobial Resistance*****, 17, 285–291.**
59. **Francino, M. P. (2016). Antibiotics and the human gut microbiome: Dysbioses and accumulation of resistances.** ***Frontiers in Microbiology*****, 6, 1543.**
60. **Frost, L. S., Leplae, R., Summers, A. O., & Toussaint, A. (2005). Mobile genetic elements: The agents of open source evolution.** ***Nature Reviews Microbiology*****, 3(9), 722–732.**
61. **Ghosh, T. S., Gupta, S. S., Nair, G. B., & Mande, S. S. (2013). In silico analysis of antibiotic resistance genes in the gut microbiome of humans.** ***Journal of Antimicrobial Chemotherapy*****, 68(2), 263–271.**
62. **Hall, C. W., & Mah, T. F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria.** ***FEMS Microbiology Reviews*****, 41(3), 276–301.**
63. **Kolpin, D. W., Furlong, E. T., Meyer, M. T., et al. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance.** ***Environmental Science & Technology*****, 36(6), 1202–1211.**
64. **Kurenbach, B., Marjoshi, D., Amábile-Cuevas, C. F., et al. (2015). Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in Escherichia coli and Salmonella enterica serovar Typhimurium.** ***mBio*****, 6(2), e00009-15.**
65. **Martínez, J. L. (2009). Environmental pollution by antibiotics and by antibiotic resistance determinants.** ***Environmental Pollution*****, 157(11), 2893–2902.**
66. **Maurice, C. F., Haiser, H. J., & Turnbaugh, P. J. (2013). Xenobiotics shape the physiology and gene expression of the active human gut microbiome.** ***Cell*****, 152(1–2), 39–50.**
67. **Michael, I., Rizzo, L., McArdell, C. S., et al. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review.** ***Science of the Total Environment*****, 447, 345–360.**
68. **Nikaido, H. (2009). Multidrug resistance in bacteria.** ***Annual Review of Biochemistry*****, 78, 119–146.**

69. **Pallecchi, L., Lucchetti, C., Bartoloni, A., et al. (2007). Population structure and resistance genes in antibiotic-resistant bacteria from a remote community with minimal antibiotic exposure. *Antimicrobial Agents and Chemotherapy*, 51(8), 2720–2725.**
70. **Pärnänen, K. M. M., Narciso-da-Rocha, C., Kneis, D., et al. (2019). Antibiotic resistance in European wastewater treatment plants mirrors the pattern of clinical antibiotic resistance prevalence. *Science Advances*, 5(3), eaau9124.**
71. **Skandalis, N., et al. (2021). Environmental pollutants and antibiotic resistance: A review. *Environmental Pollution*, 268(Pt A), 115642.**
72. **Sommer, M. O. A., Dantas, G., & Church, G. M. (2009). Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science*, 325(5944), 1128–1131.**
73. **Wales, A. D., & Davies, R. H. (2015). Co-selection of resistance to antibiotics, biocides and heavy metals, and its relevance to foodborne pathogens. *Antimicrobial Resistance and Infection Control*, 4, 4.**

74. **Adhikary, S., Chakraborty, R., & Mukherjee, S. (2024). Metal binding and chelation in microbial communities: Implications for gut microbiota. *Journal of Environmental Microbiology*, 30(2), 123–135.**
75. **Arneth, B. M., & Shams, A. (2017). The gut-brain axis in neurodevelopmental disorders: A review. *Neuroscience Bulletin*, 33(2), 131–137.**
76. **Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., ... & Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences*, 101(44), 15718–15723.**
77. **Baker-Austin, C., Wright, M. S., Stepanauskas, R., & McArthur, J. V. (2006). Co-selection of antibiotic and metal resistance. *Trends in Microbiology*, 14(4), 176–182.**
78. **Baumgart, D. C., & Carding, S. R. (2007). Inflammatory bowel disease: Cause and immunobiology. *The Lancet*, 369(9573), 1627–1640.**
79. **Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell*, 157(1), 121–141.**
80. **Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. G. J. (2018). Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiology Reviews*, 42(1), fux053.**
81. **Berg, J., Tom-Petersen, A., & Nybroe, O. (2005). Copper amendment of agricultural soil selects for bacterial antibiotic resistance in the field. *Letters in Applied Microbiology*, 40(2), 146–151.**
82. **Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. V. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, 13(1), 42–51.**

83. **Breton, J., Massart, S., Vandamme, P., De Brandt, E., Pot, B., & Foligné, B. (2013). Ecotoxicology inside the gut: Impact of heavy metals on the mouse microbiome. *BMC Pharmacology and Toxicology*, 14(1), 62.**
84. **Canfora, E. E., Jocken, J. W. E., & Blaak, E. E. (2015). Short-chain fatty acids in control of body weight and insulin sensitivity. *Nature Reviews Endocrinology*, 11(10), 577–591.**
85. **Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., ... & Delzenne, N. M. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*, 56(7), 1761–1772.**
86. **Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M., & Owen, L. J. (2015). Dysbiosis of the gut microbiota in disease. *Microbial Ecology in Health and Disease*, 26(1), 26191.**
87. **Carroll, I. M., Ringel-Kulka, T., Siddle, J. P., Klaenhammer, T. R., & Ringel, Y. (2010). Characterization of the fecal microbiota using high-throughput sequencing reveals a stable microbial community during storage. *PloS One*, 5(10), e13213.**
88. **Castellani, V. (2025). Gut microbiota and cardiovascular diseases: The role of dysbiosis in atherosclerosis. *Cardiovascular Research*, 121(3), 345–356.**
89. **Chowdhury, S., Routh, J., & Paul, D. (2016). Environmental reservoirs of antibiotic resistance genes. *Environmental Science and Pollution Research*, 23(1), 1–12.**
90. **Claus, S. P., Guillaume, C., & Ellero-Simatos, S. (2016). The gut microbiota: A major player in the toxicity of environmental pollutants? *NPJ Biofilms and Microbiomes*, 2, 16003.**
91. **Cusumano, G., & Rossolini, G. M. (2025). The emergence of antibiotic resistance: A global health concern. *Journal of Global Antimicrobial Resistance*, 23, 1–5.**
92. **De La Cochetière, M. F., Durand, T., Lepage, P., Bourreille, A., Galmiche, J. P., & Doré, J. (2005). Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge. *Journal of Clinical Microbiology*, 43(11), 5588–5592.**
93. **Di Vincenzo, F., Marasco, G., & Festi, D. (2024). Gut microbiota and autoimmune diseases: A review. *Autoimmunity Reviews*, 23(1), 102–110.**
94. **Doucet-Populaire, F., Trieu-Cuot, P., Dosbaa, I., Andremont, A., Courvalin, P., & Duval, J. (1991). Inducible transfer of conjugative transposon Tn1545 from Enterococcus faecalis to Listeria monocytogenes in the digestive tracts of gnotobiotic mice. *Antimicrobial Agents and Chemotherapy*, 35(1), 185–187.**
95. **Faleye, A. C., Olayemi, A. O., & Adeniji, J. A. (2019). Environmental reservoirs of antibiotic resistance genes: A review. *African Journal of Clinical and Experimental Microbiology*, 20(3), 231–240.**
96. **Francino, M. P. (2016). Antibiotics and the human gut microbiome: Dysbioses and accumulation of resistances. *Frontiers in Microbiology*, 6, 1543.**
97. **Frost, L. S., Leplae, R., Summers, A. O., & Toussaint, A. (2005). Mobile genetic elements: The agents of open source evolution. *Nature Reviews Microbiology*, 3(9), 722–732.**
98. **Ghosh, T. S., Gupta, S. S., Nair, G. B., & Mande, S. S. (2013). In silico analysis of antibiotic resistance genes in the human gut microbiome. *PloS One*, 8(12), e83823.**
99. **Hall, C. W., & Mah, T. F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews*, 41(3), 276–301.**
100. **Jomova, K., & Valko, M. (2011). Advances in metal-induced oxidative stress and human disease. *Toxicology*, 283(2–3), 65–87.**
101. **Jones, B. V., Begley, M., Hill, C., Gahan, C. G., & Marchesi, J. R. (2008). Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proceedings of the National Academy of Sciences*, 105(36), 13580–13585.**
102. **Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B., & Buxton, H. T. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. *Environmental Science & Technology*, 36(6), 1202–1211.**
103. **Koren, O., Knights, D., Gonzalez, A., Waldron, L., Segata, N., Knight, R., ... & Ley, R. E. (2011). A guide to enterotypes across the human body: Meta-analysis of microbial community structures in human microbiome datasets. *PloS Computational Biology*, 7(1), e1002355.**

104. **Kurenbach, B., Marx, K., & Hill, A. M. (2015). Non-antibiotic pharmaceuticals can induce antibiotic resistance. *Journal of Antimicrobial Chemotherapy*, 70(8), 2225–2233.**
105. **Maurice, C. F., Haiser, H. J., & Turnbaugh, P. J. (2013). Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell*, 152(1–2), 39–50.**
106. **Bhattacharya, P., Mukherjee, A. B., Jacks, G., & Nordqvist, S. (2007). Groundwater arsenic contamination in India: vulnerability and scope for remedy. *Water Resources Development*, 23(1), 79–92.**
107. **Chakraborti, D., Rahman, M. M., Das, B., Murrill, M., Dey, S., Mukherjee, S. C., ... & Quamruzzaman, Q. (2004). Groundwater arsenic contamination and its health effects in the Ganga-Meghna-Brahmaputra plain. *Journal of Environmental Monitoring*, 6(6), 74N–83N.**
108. **Chi, L., Bian, X., Gao, B., Tu, P., Lai, Y., Ru, H., & Lu, K. (2018). The Effects of an Environmentally Relevant Level of Arsenic on the Gut Microbiota and Its Functional Metagenome. *Toxicological Sciences*, 164(2), 351–361.**
109. **Hughes, M. F. (2002). Arsenic toxicity and potential mechanisms of action. *Toxicology Letters*, 133(1), 1–16.**
110. **Kümmerer, K. (2009). Antibiotics in the aquatic environment–A review–Part I. *Chemosphere*, 75(4), 417–434.**
111. **Kumar, M., Puri, A. (2009). A review of permissible limits of drinking water. *Indian Journal of Occupational and Environmental Medicine*, 13(1), 6–10.**
112. **Lu, K., Abo, R. P., Schlieper, K. A., Graffam, M. E., Levine, S., Wishnok, J. S., ... & Fox, J. G. (2014). Arsenic exposure perturbs the gut microbiome and its metabolic profile in mice: an integrated metagenomics and metabolomics analysis. *Environmental Health Perspectives*, 122(3), 284–291.**
113. **Mazumder, D. N. G. (2008). Chronic arsenic toxicity & human health. *Indian Journal of Medical Research*, 128(4), 436–447.**
114. **Naujokas, M. F., Anderson, B., Ahsan, H., Vasken Aposhian, H., Graziano, J. H., Thompson, C., & Suk, W. A. (2013). The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. *Environmental Health Perspectives*, 121(3), 295–302.**
115. **Nickson, R. T., McArthur, J. M., Ravenscroft, P., Burgess, W. G., & Ahmed, K. M. (2000). Mechanism of arsenic release to groundwater, Bangladesh and West Bengal. *Applied Geochemistry*, 15(4), 403–413.**
116. **Seiler, C., & Berendonk, T. U. (2012). Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Frontiers in Microbiology*, 3, 399.**
117. **Shankar, S., Shanker, U., & Shikha. (2014). Arsenic contamination of groundwater: A review of sources, prevalence, health risks, and strategies for mitigation. *The Scientific World Journal*, 2014, Article ID 304524.**
118. **Smedley, P. L., & Kinniburgh, D. G. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry*, 17(5), 517–568.**
119. **Smith, A. H., Lingas, E. O., & Rahman, M. (2000). Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bulletin of the World Health Organization*, 78(9), 1093–1103.**
120. **Vats, P., Sharma, A., Sood, R., & Beniwal, V. (2022). Occurrence and distribution of antibiotic resistance genes in Indian aquatic environments: A review. *Environmental Pollution*, 292, 118388.**
121. **Zhu, Y. G., Johnson, T. A., Su, J. Q., Qiao, M., Guo, G. X., Stedtfeld, R. D., ... & Tiedje, J. M. (2013). Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proceedings of the National Academy of Sciences*, 110(9), 3435–3440.**
122. **Alam, M., Singh, S. K., & Sharma, A. (2022). *Antibiotic resistance patterns in arsenic-contaminated regions of India*. Environmental Microbiology Reports.**
123. **Berg, J., Tom-Petersen, A., & Nybroe, O. (2005). Copper amendment of agricultural soil selects for bacterial antibiotic resistance in the field. *Letters in Applied Microbiology, 40*(2), 146–151.**
124. **Bhattacharya, A., Gupta, A., & Ghosh, A. (2000). Metal resistance and antibiotic resistance in bacteria from activated sludge. *Bulletin of Environmental Contamination and Toxicology, 64*(4), 488–495.**
125. **Bruins, M. R., Kapil, S., & Oehme, F. W. (2000). Microbial resistance to metals in the environment. *Ecotoxicology and Environmental Safety, 45*(3), 198–207.**
126. **Cardetti, M., Lin, J. H., & Cai, L. (2020). Antibiotic-induced dysbiosis and its systemic impact. *Journal of Clinical Microbiology and Infection, 26*(9), 1125–1131.**
127. **Chouhan, R., & Flora, S. J. S. (2010). Arsenic and fluoride: Two major ground water pollutants. *Indian Journal of Experimental Biology, 48*(7), 666–678.**
128. **Giller, K. E., Witter, E., & McGrath, S. P. (1998). Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. *Soil Biology and Biochemistry, 30*(10–11), 1389–1414.**
129. **Helaly, F. M., Roshdy, N. M., & Al-Kahtani, M. (2019). Antibiotics and neurotoxicity: Association with psychiatric disorders. *Neuroscience Bulletin, 35*(3), 366–377.**
130. **Klein, E. Y., Van Boeckel, T. P., Martinez, E. M., et al. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academy of Sciences, 115*(15), E3463–E3470.**
131. **Knapp, C. W., Dolfing, J., Ehlert, P. A. I., & Graham, D. W. (2010). Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. *Environmental Science & Technology, 44*(2), 580–587.**
132. **Kholodov, A. S., Kravchenko, I. K., Tikhonovich, I. A., & Smirnov, A. N. (2021). Role of the microbiome in metal homeostasis and toxicity. *Microbiome Research Reports, 3*(2), 45–56.**
133. **Kümmerer, K. (2009). Antibiotics in the aquatic environment–A review–Part I. *Chemosphere, 75*(4), 417–434.**
134. **Lu, K., Abo, R. P., Schlieper, K. A., et al. (2014). Arsenic exposure perturbs the gut microbiome and its metabolic profile in mice: An integrated metagenomics and metabolomics analysis. *Environmental Health Perspectives, 122*(3), 284–291.**
135. **Moore, B. (1960). Heavy metal resistance in bacteria. *Nature, 187*, 1179–1180.**
136. **Morita, T., et al. (2019). Antibiotic resistance gene dynamics under arsenic pressure in gut microbiota. *Environmental Science & Technology Letters, 6*(9), 565–572.**
137. **Parrone, D., et al. (2020). Arsenic contamination in groundwater and its impact on human health in South Asia. *Science of the Total Environment, 730*, 139188.**
138. **Pathak, S. P., & Gopal, K. (2005). Occurrence of antibiotic and metal resistance in bacteria from organs of river fish. *Environmental Research, 98*(1), 100–103.**
139. **Peltier, E., Vincent, J. M., Finn, C., & Graham, D. W. (2010). Zinc-induced antibiotic resistance in a model wastewater treatment system. *Environmental Science & Technology, 44*(19), 7390–7396.**
140. **Richmond, M. H., & John, M. (1964). Multiple antibiotic resistance due to R factors. *British Medical Journal, 1*(5383), 238.**
141. **Seiler, C., & Berendonk, T. U. (2012). Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Frontiers in Microbiology, 3*, 399.**
142. **Singh, S., Sinha, A., & Gupta, R. (2023). Industrial arsenic exposure causes gut dysbiosis in murine models. *Environmental Toxicology and Pharmacology, 95*, 104113.**

143. **Stepanauskas, R., Glenn, T. C., Jagoe, C. H., & Tuckfield, R. C. (2006). Elevated microbial tolerance to metals and antibiotics in mercury-polluted river sediments. *Environmental Science & Technology, 40*(23), 7077–7082.**
144. **Tang, S., Wang, H., & Liu, X. (2020). Fecal bacteria as a proxy for intestinal microbiota in heavy metal exposure studies. *Toxicology Reports, 7*, 302–310.**
145. **Van Boeckel, T. P., Brower, C., Gilbert, M., et al. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences, 112*(18), 5649–5654.**
146. **Wu, N., Yang, X., Zhang, R., et al. (2020). Dysbiosis of gut microbiota is associated with arsenic toxicity and altered host metabolism. *Environmental Pollution, 261*, 114140.**
147. **Yang, Y., Li, B., Zou, S., Fang, H. H. P., & Zhang, T. (2019). Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach. *Water Research, 62*, 97–106.**
148. **Zhang, L., Gu, X., Zhao, X., et al. (2020). Arsenic induces antibiotic resistance gene proliferation in the gut microbiome. *Environmental International, 136*, 105448.**
149. **AAT Bioquest. (2023). *LD50 Dosage Calculator*.**
150. **Bhatia, P., Singh, A., & Shukla, R. K. (2022). Ethical euthanasia in laboratory animals: An overview. *Journal of Pharmacological and Toxicological Methods*, *113*, 107116.**
151. **Central Ground Water Board. (2009). *Ground Water Quality in Shallow Aquifers of India*. Ministry of Water Resources, Government of India.**
152. **Datta, D. V., Saha, T., Banerjee, R., & Mukherjee, S. (2014). Assessment of arsenic exposure from groundwater in West Bengal: A case study. *Environmental Geochemistry and Health*, *36*(3), 441–451.**
153. **Kumar Sharma, A., Kumar, S., & Tripathi, P. (2021). Arsenic-induced toxicity and its mitigation by herbal intervention: A review. *Environmental Science and Pollution Research*, *28*(5), 5226–5245.**
154. **Kumar Sharma, A. (2006). Toxicological effects of arsenic on laboratory rodents. *Toxicology and Industrial Health*, *22*(9), 381–390.**
155. **Mehrzadi, S., Hosseinzadeh, H., & Mohammadpour, A. H. (2021). Protective effects of natural antioxidants against arsenic-induced toxicity: A review. Drug and Chemical Toxicology, 44(1), 1–9.**
156. **Banerjee, G., Ray, A. K., & Das, D. (2011). Isolation and characterization of arsenic-resistant bacteria from contaminated groundwater of West Bengal, India. Ecotoxicology and Environmental Safety, 74(4), 628–635.**
157. **Duranti, S., Lugli, G. A., Mancabelli, L., Armanini, F., Turroni, F., James, K., ... & Ventura, M. (2017). Evaluation of the probiotic potential of a Bifidobacterium strain isolated from a centenarian. Nutrition, 38, 28–36.**
158. **Maden, M., Altun, S., & Aslan, H. (2011). Antibiotic resistance and plasmid profiles of Escherichia coli isolated from the urine samples of patients in a university hospital in Turkey. African Journal of Microbiology Research, 5(21), 3432–3437.**
159. **National Committee for Clinical Laboratory Standards (NCCLS). (2011). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. Clinical and Laboratory Standards Institute (CLSI) document M100–S21.**
160. **Shakya, P., Barrett, P., Diwan, V., Marothi, Y., Shah, H., Chhari, N., ... & Tamhankar, A. J. (2012). Antibiotic resistance among Escherichia coli isolates from stool samples of children aged 3 to 14 years from Ujjain, India. BMC Infectious Diseases, 13(1), 1-7.**
161. **Thakurta, R., Ghosh, A. N., & Saha, S. K. (2007). A study on antimicrobial susceptibility pattern of Pseudomonas aeruginosa isolated from clinical samples in a tertiary care hospital. Indian Medical Gazette, 141, 57–60.**

PLAGIARISM REPORT

BARTA MUKHERJEE

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